# Blocking Protein Secretion with Novel Small Molecule Inhibitors of Sec61 Represents a Potential Treatment Strategy Against Hematologic Malignancies

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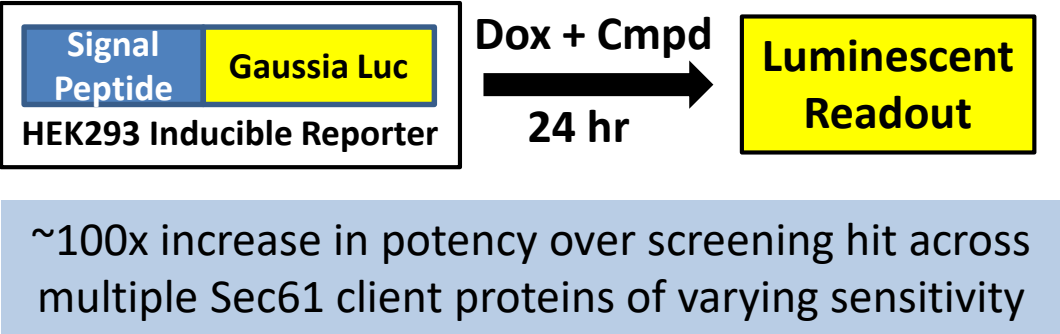
ASH 2019 – Abstract #408

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Targeting Protein Homeostasis by Blocking Sec61 Dependent Co-translational Translocation into the ER
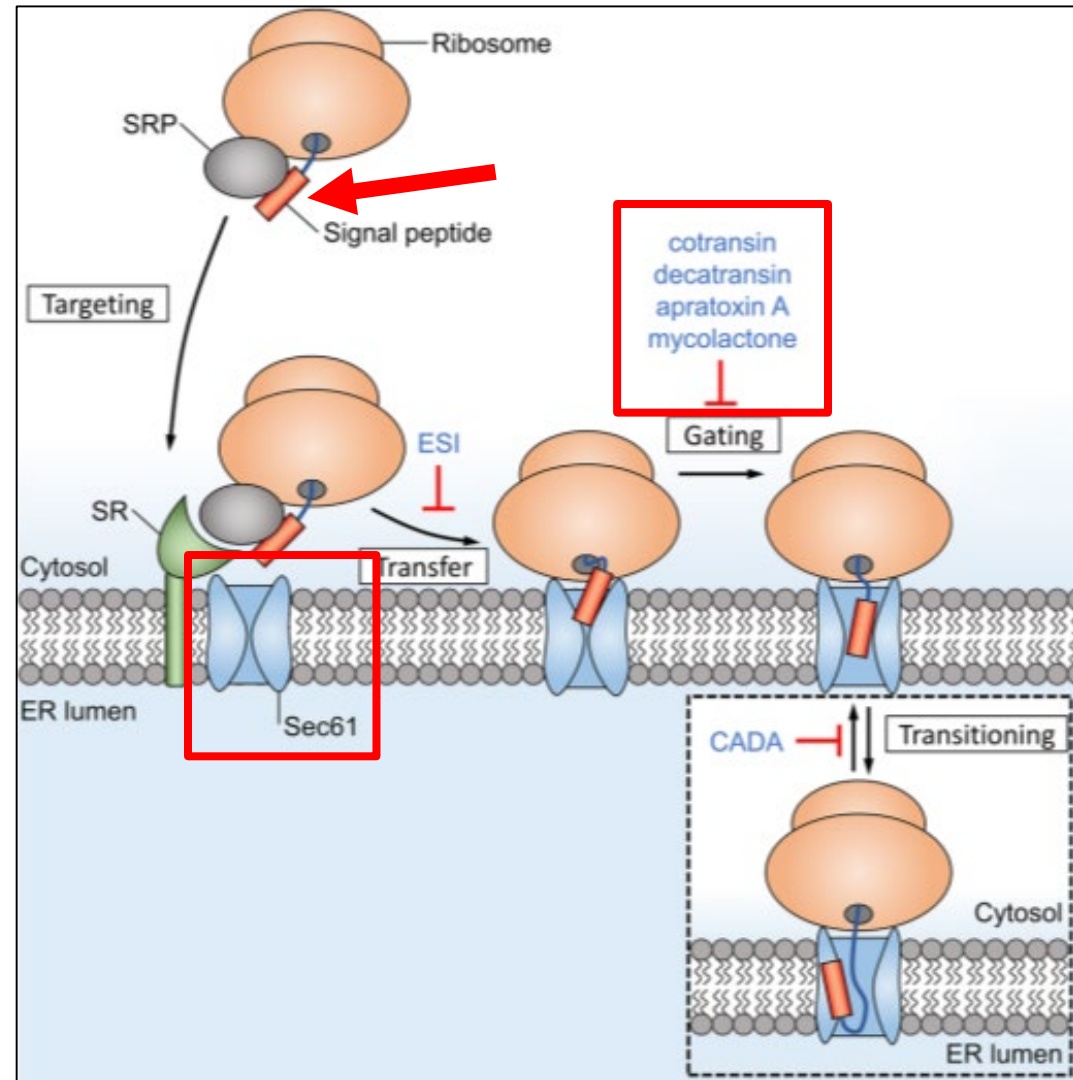
- Sec61 mediates co-translational translocation of nearly all secreted and transmembrane "client" proteins into the endoplasmic reticulum

- Client proteins targeted to Sec61 through recognition of signal peptide unique to each protein – opportunity for broad or selective inhibition of clients

- Previously described Sec61 inhibitors demonstrate anti-tumor activity but lack adequate pharmaceutical properties and/or tolerability for further development

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Discovery of Potent Inhibitors of Sec61 Dependent Secretion

Luciferase Reporter Screen of Sec61 Client Proteins

Signal Peptide	Gaussia Luc

HEK293 Inducible Reporter

Dox + Cmpd
24 hr
→
Luminescent Readout

~100x increase in potency over screening hit across multiple Sec61 client proteins of varying sensitivity

IC_{50} (nM)	PD-1	IL-2	TNF-α	HER3	Prolactin
KZR-152	995	5150	>25000	2738	>25000
KZR-834	9	40	269	31	>25000
KZR-261	7	21	197	20	>25000

On-Target Confirmation of Lead Series
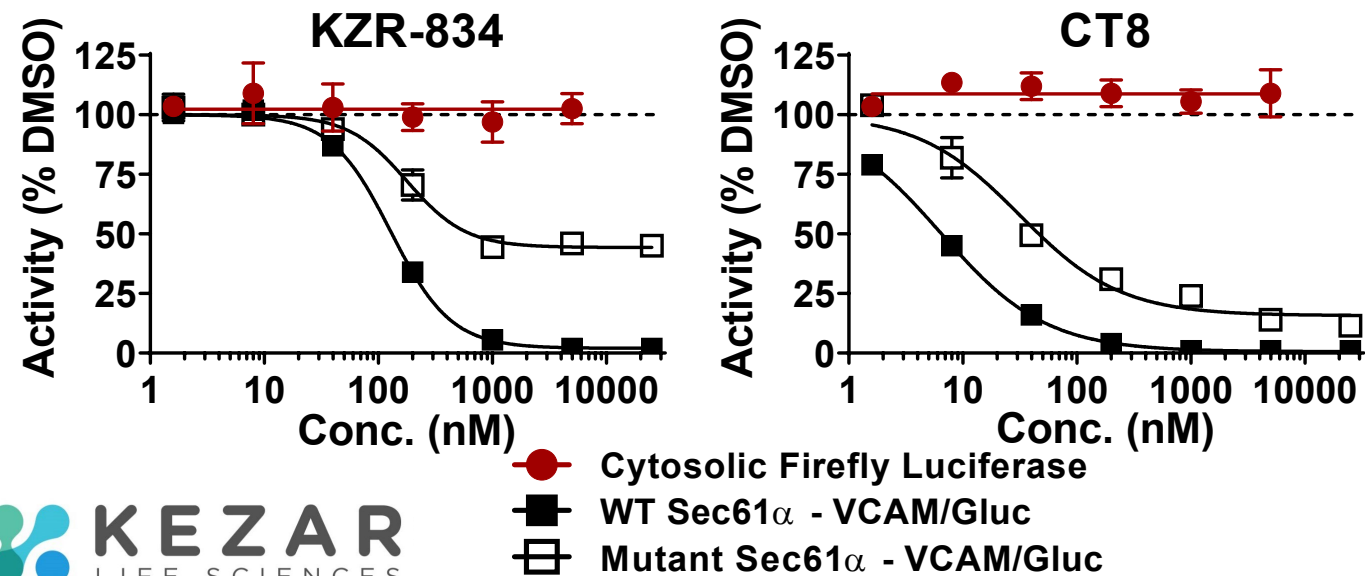
No effect on Sec61 independent expression of firefly luciferase

Decreased activity against Sec61 (R66I) mutant
- Similar profile to validated Sec61 inhibitor CT8[1]

Legend:
- ● Cytosolic Firefly Luciferase
- ■ WT Sec61α - VCAM/Gluc
- □ Mutant Sec61α - VCAM/Gluc

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[1]MacKinnon et al. *eLife*. 2014; 3:e01483

KZR-834 Blocks Expression of Therapeutically Relevant Targets in Expanded Inducible Reporter Screen

Cytokines & Cytokine Receptors

Target	Compound 834	Compound 261
TNFR1		
TNFR2		
IL-17A		
IL-17RA		
IL-23A		
IL-12B		
IL-23R		
IL-6		
IL-6R		
GP130		
IL-1R1		
IL-1R2		
IL-1RA		
IL-2RA		
IFN-A1		
IFN-A2		
IFN-A4		
IFN-B		
IFN-G		
IFNAR1		
IFNAR2		

Immune Checkpoints

Target	Compound 834	Compound 261
CTLA-4		
PD-1		
PD-L1		
LAG3		
TIM3		
TIGIT		
CD96		
VISTA		
B7H3		
CD73		
CD47		

Oncogenic Factors

Target	Compound 834	Compound 261
PDGFRa		
VGFR2		
IL-7R		
EGFR		
VEGF		
HER3		

Meyer et al., ASH 2019, Abstract# 805
Session 605 – Mon, Dec 9th 4:30pm

IC$_{50}$ (nM)	
1	
100	
250	
500	
750	
900	
>1000	

Low nanomolar potency against many targets of potential therapeutic interest and currently approved biologics

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KZR-834 has Minimal Effect on Total Sec61 Client Expression

Global Proteomic Analysis of KZR-834 Treated Myeloma Cells (24hr)

Total Sec61 Clients Identified

Sec61 Clients Downregulated >1.5 Fold

211   825   239

H929   U266

36   17   59

H929   U266
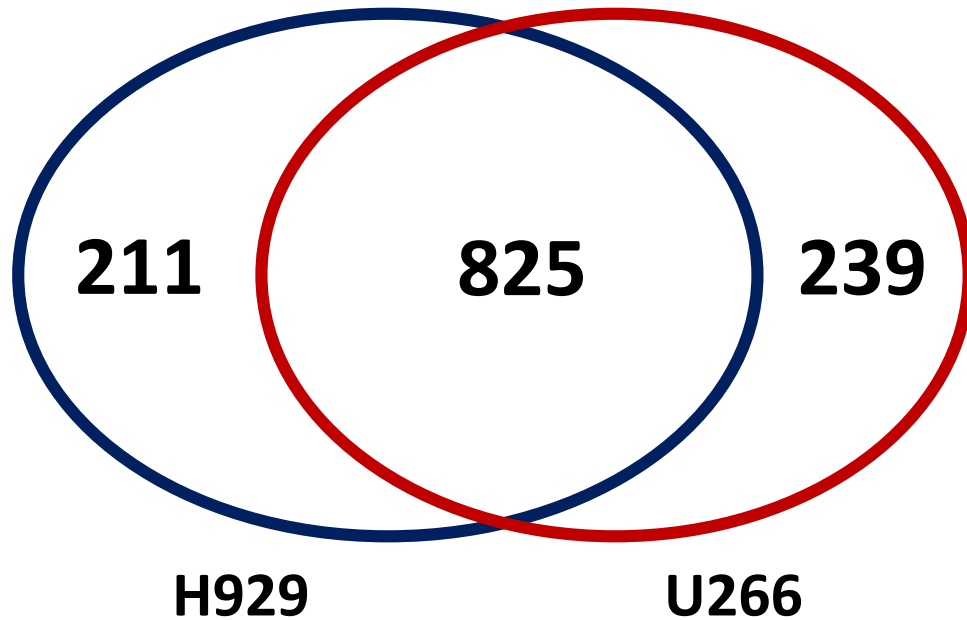
~1/7th of all predicted Sec61 clients detected in measurable quantities in two myeloma cell lines
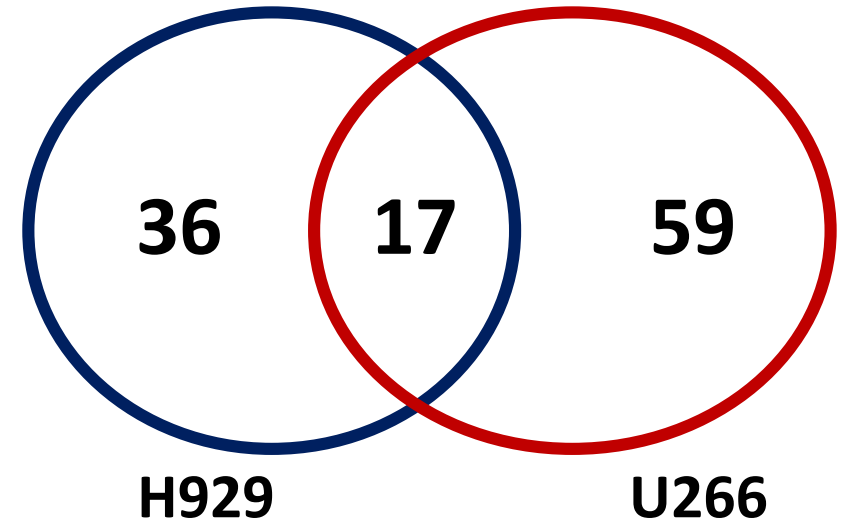
<10% of detected Sec61 client proteins downregulated >1.5 fold after KZR-834 treatment (250nM)

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Qian et al., ASH 2019, Abstract# 2076

KZR-834 Exhibits Selective Anti-Cancer Cytotoxicity
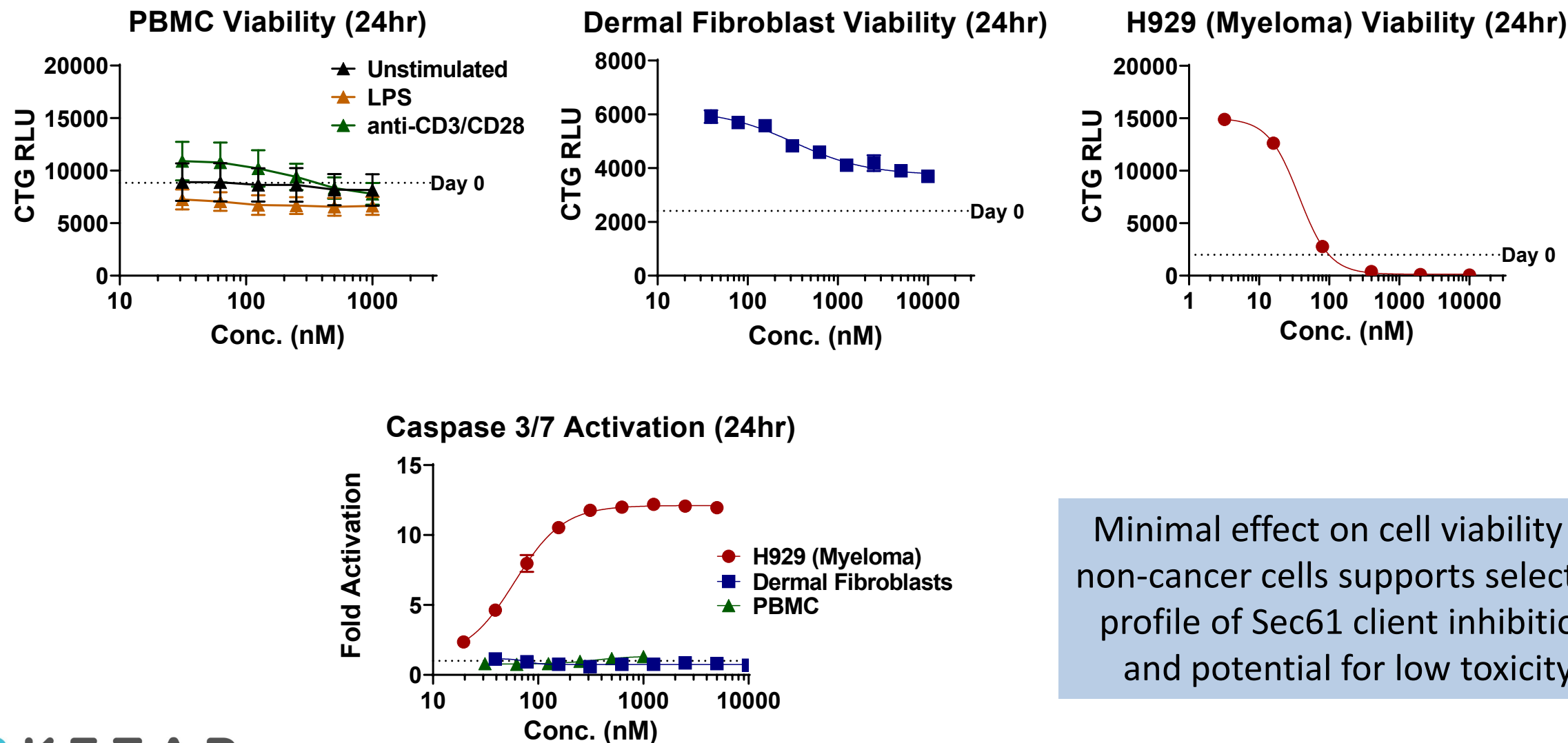
Minimal effect on cell viability of non-cancer cells supports selective profile of Sec61 client inhibition and potential for low toxicity

Potent Sec61 Inhibition by KZR-834 Results in Broad Anti-Tumor Activity

KZR-834 Cell Line Screen
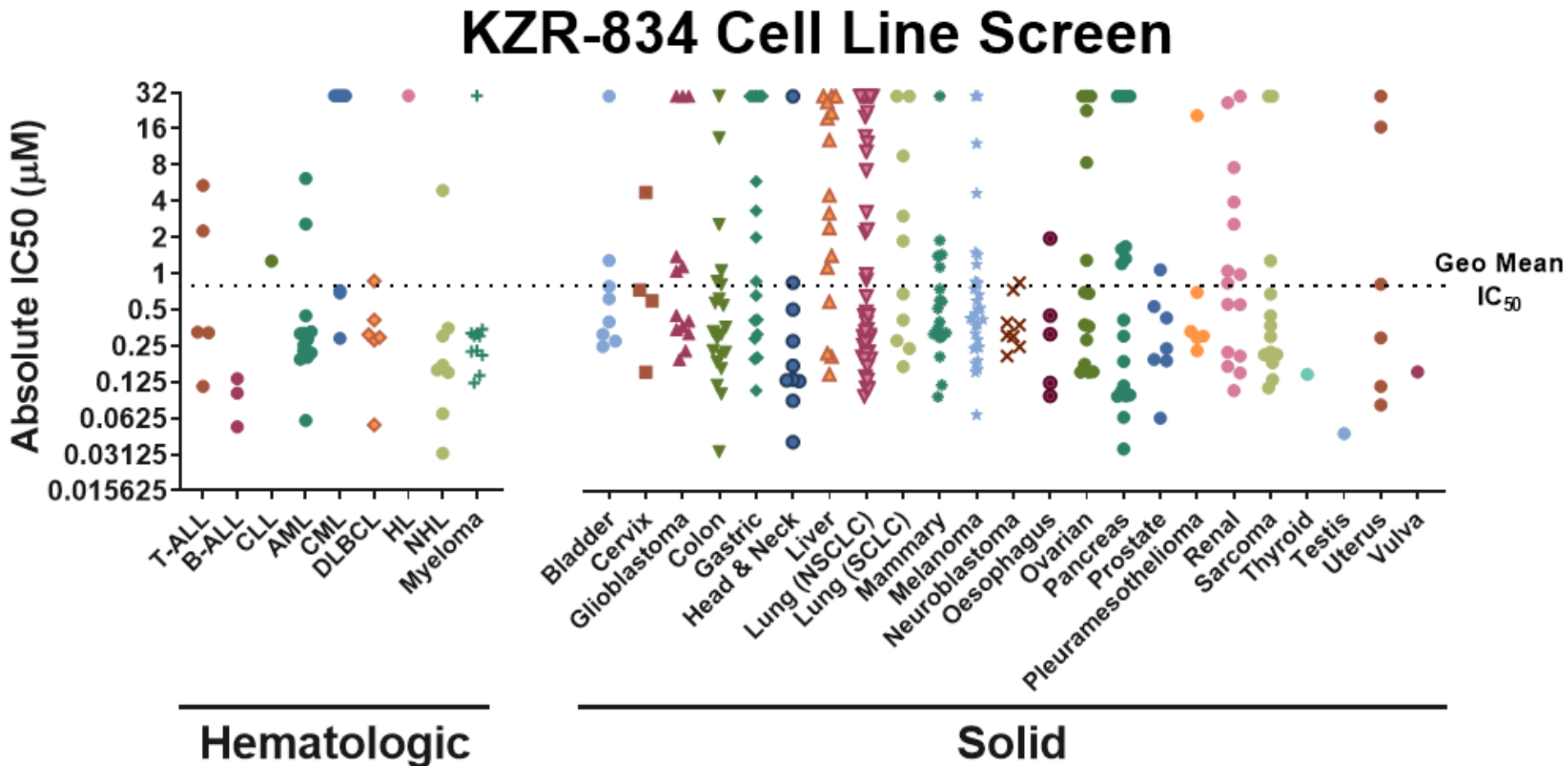
	T-ALL	B-ALL	AML	CLL	CML	DLBCL	HL	NHL	Myeloma	All (Heme + Solid)
Mean IC$_{50}$ (μM)	0.68 n=5	.09 n=3	0.36 n=14	1.27 n=1	6.57 n=8	0.28 n=6	>30 n=1	0.22 n=8	0.35 n=11	0.79 n=346

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Sec61 Blockade is Efficacious Against Multiple Heme Tumors

RL (NHL)

Vehicle
15 mg/kg KZR-261 IV QW
25 mg/kg KZR-834 IV QW

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**

Mino (MCL)

Vehicle
20 mg/kg KZR-261 IV QW
25 mg/kg KZR-834 IV QW

**

H929 (Myeloma)

Vehicle
25 mg/kg KZR-834 QW IV
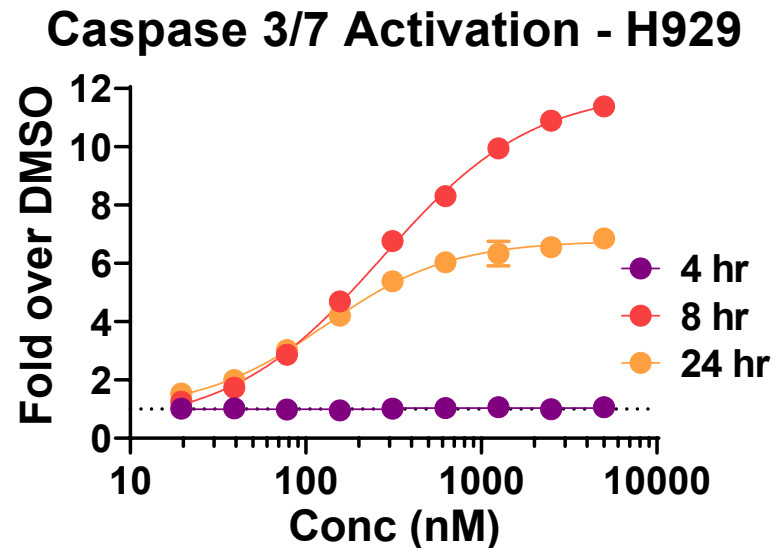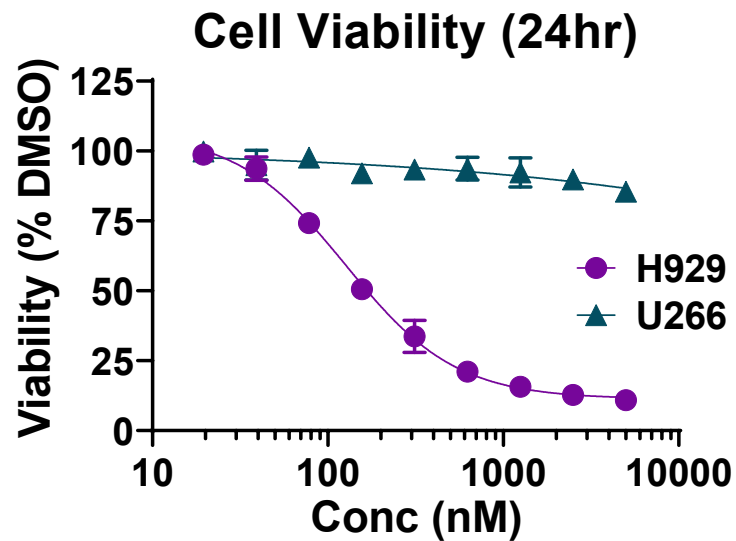15 mg/kg KZR-834 QDx2 IV

*
*
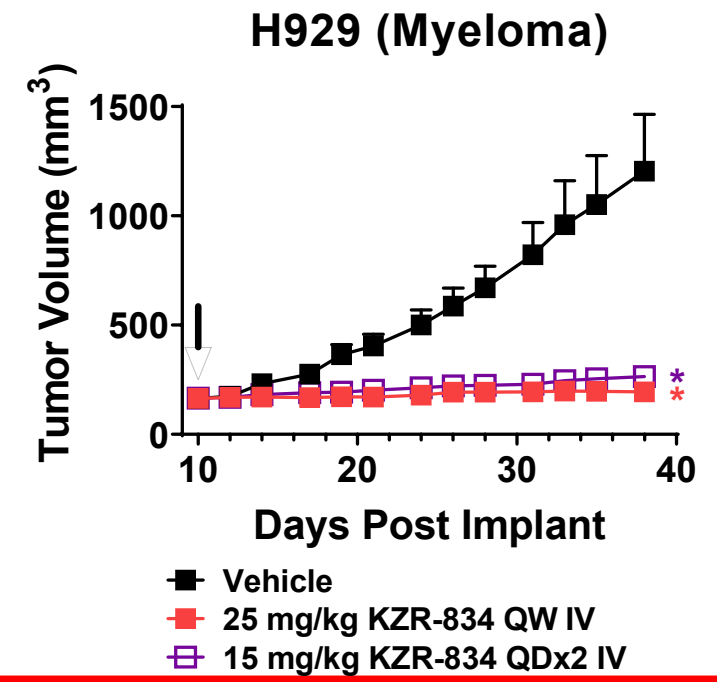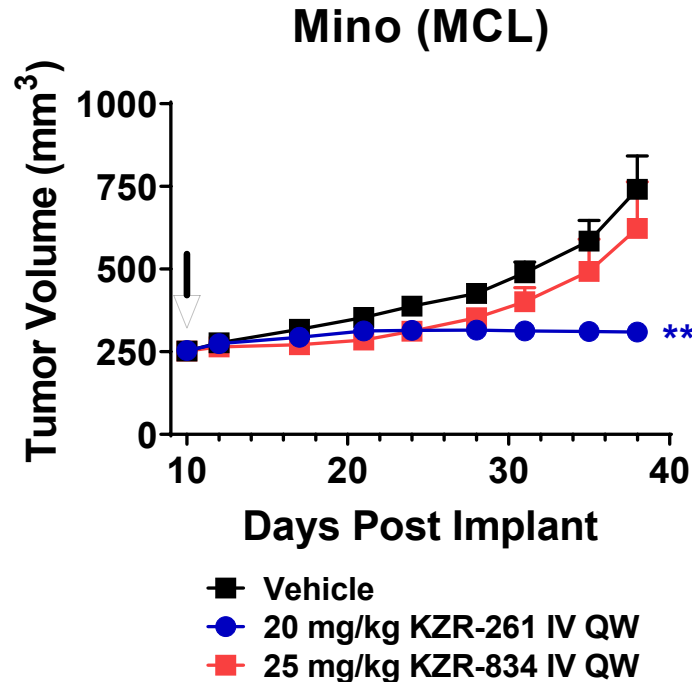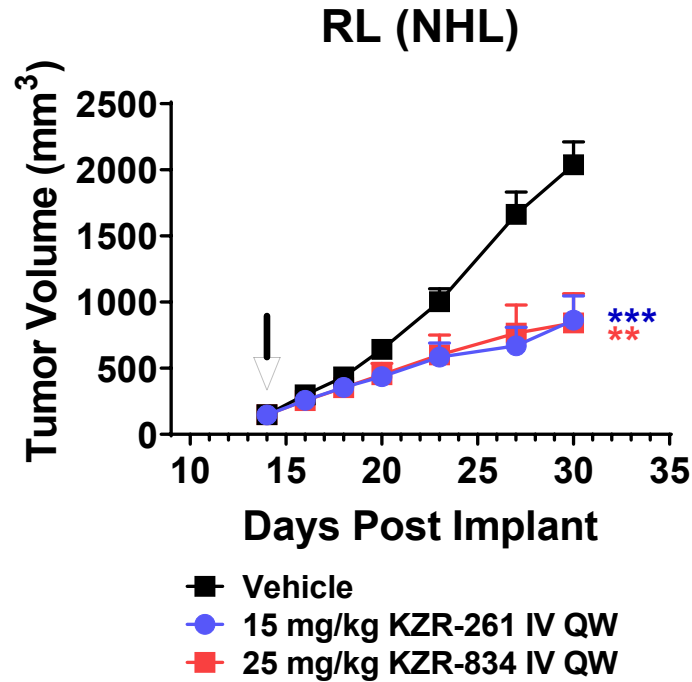
Start of Treatment

*P<0.05; **P<0.01; ***P<0.001

Significant tumor growth inhibition in myeloma and lymphoma xenograft models

Transient body weight loss <15% at efficacious doses seen only in the first week of dosing
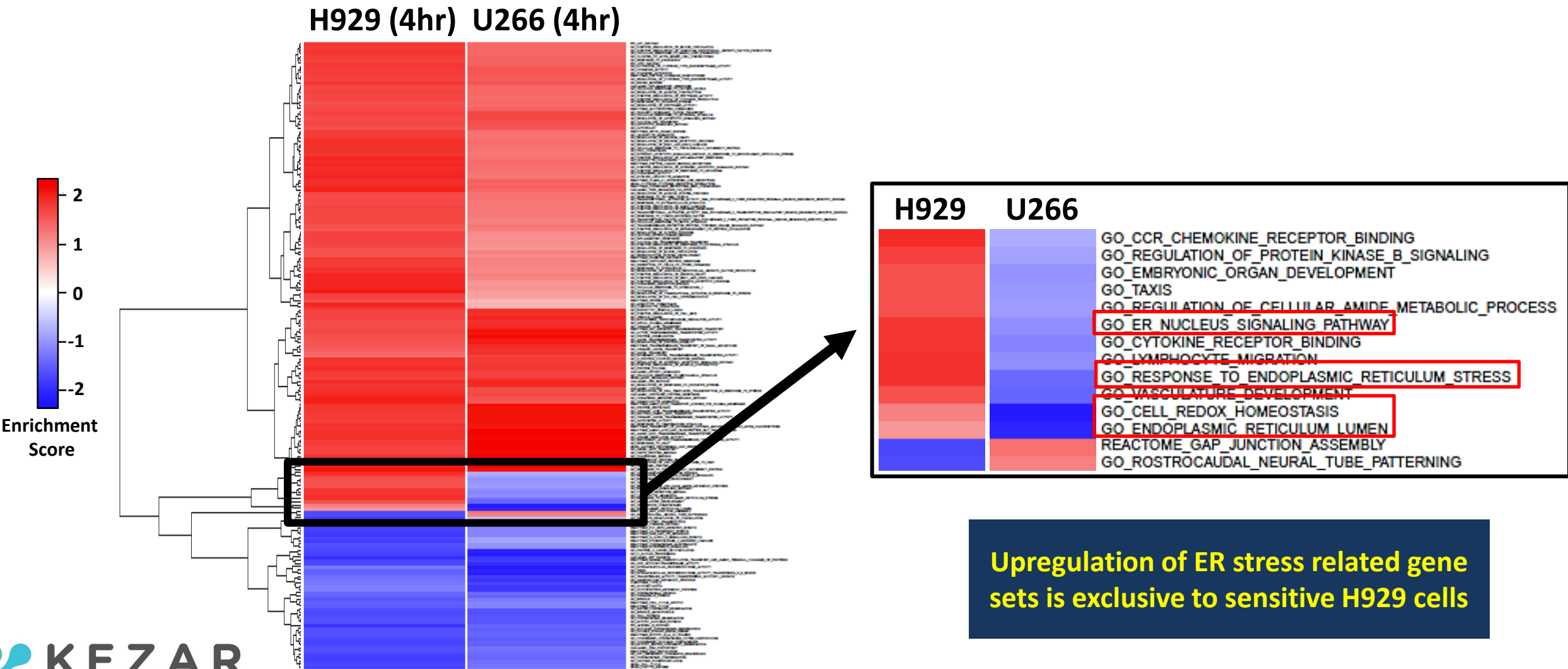
H929 (BNX) - Body Weight
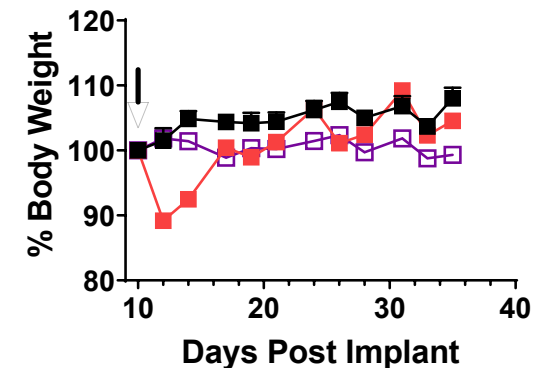
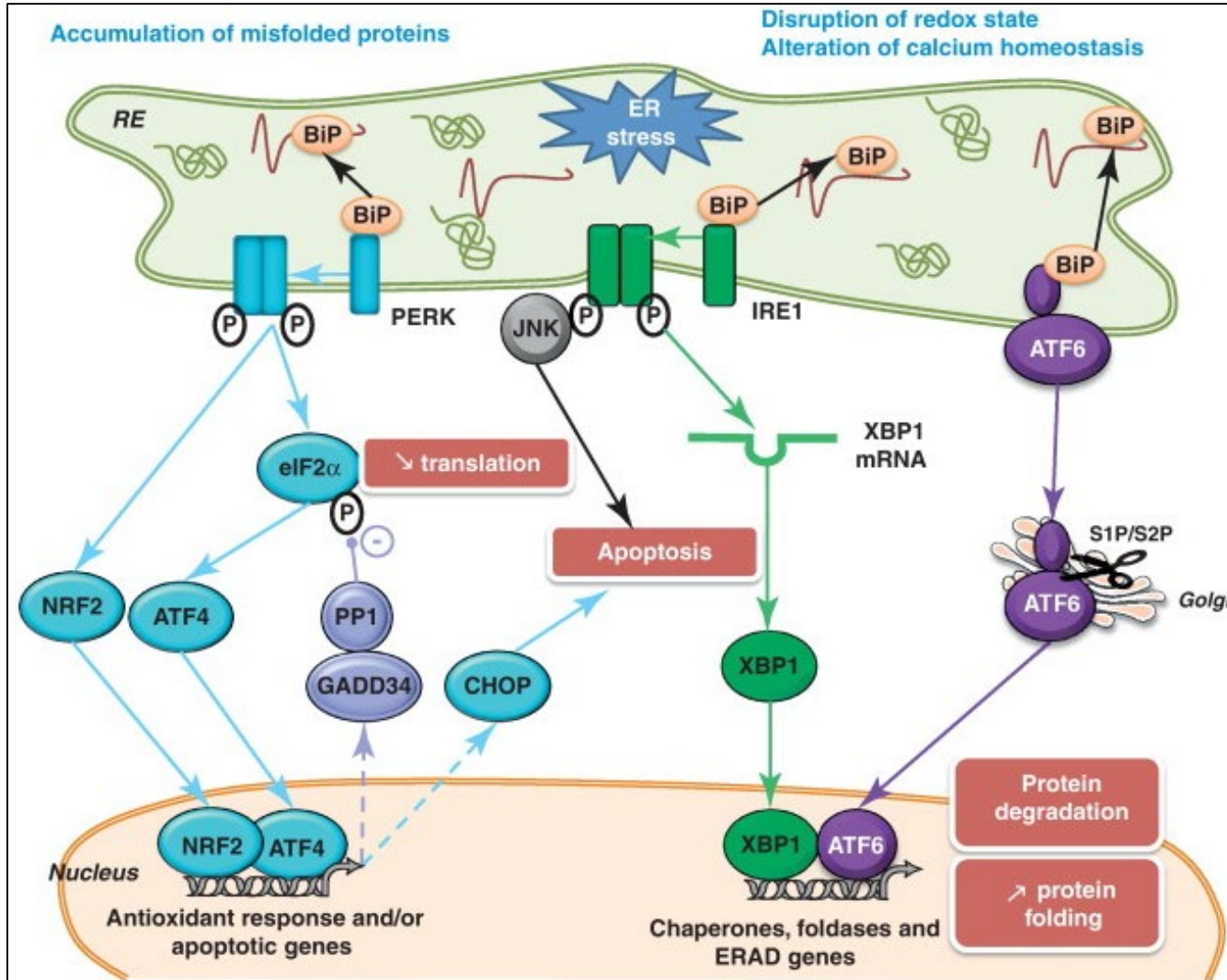# Gene Expression Profiling of Sensitive and Resistant Myeloma Cell Lines
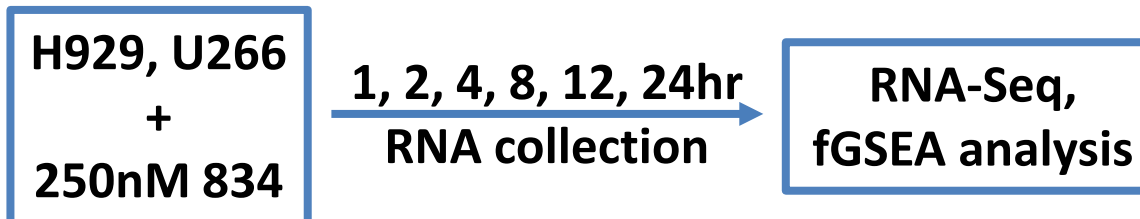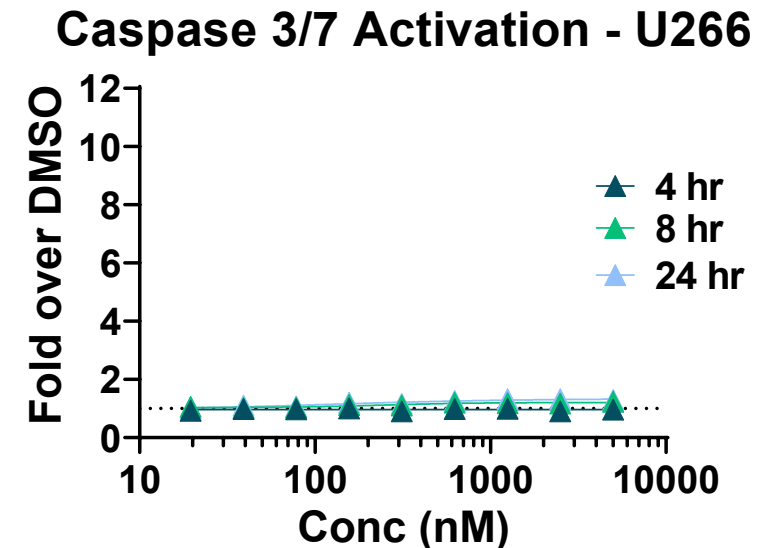
Gene Expression Profiling Reveals Differential ER Stress Response

## Gene Set Enrichment Analysis – 834 vs DMSO

Upregulation of ER stress related gene sets is exclusive to sensitive H929 cells

Activation of the Unfolded Protein Response: Background

Accumulation of misfolded proteins

Disruption of redox state
Alteration of calcium homeostasis

- 3 branches of UPR: PERK, IRE1, ATF6
- Activated upon accumulation of misfolded proteins
- Acute activation reduces protein load, increases protein folding capacity
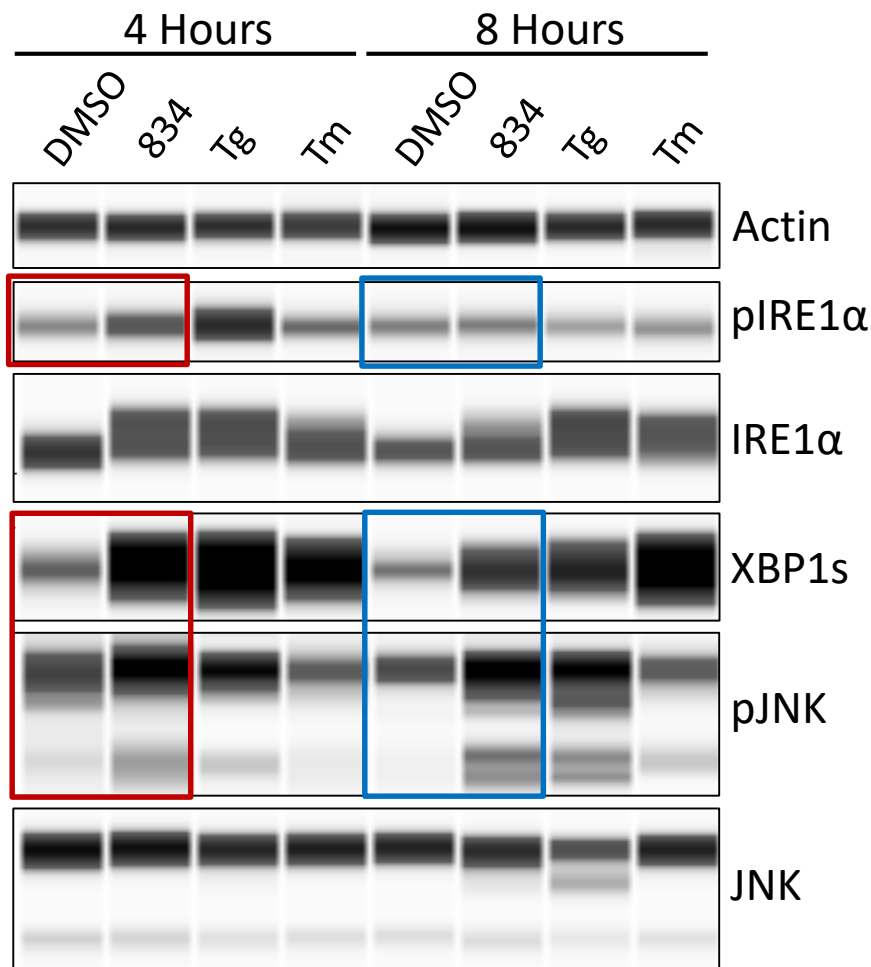- Prolonged activation can lead to cell death

Activation of the Unfolded Protein Response: KZR-834 Induces Rapid, Potent Increase in IRE1 Activity
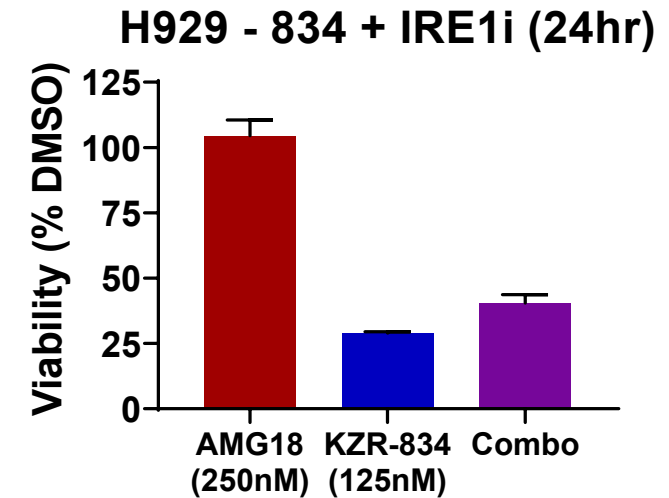

IRE1 Branch of UPR
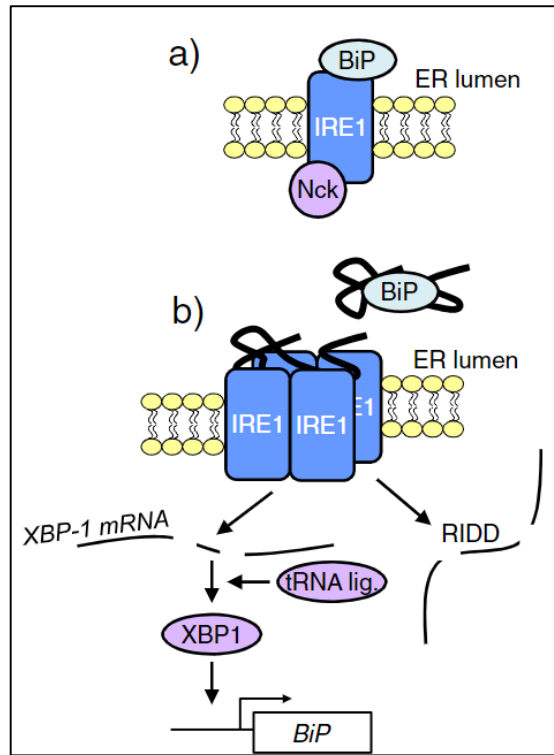
IRE1 activation (4hr) precedes detection of caspase activity (8hr)
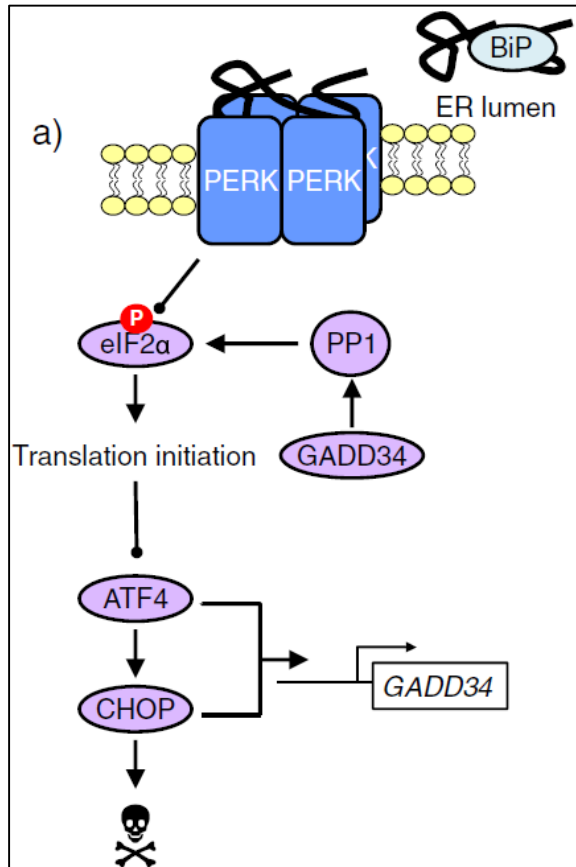
H929 (sensitive) Myeloma Cells

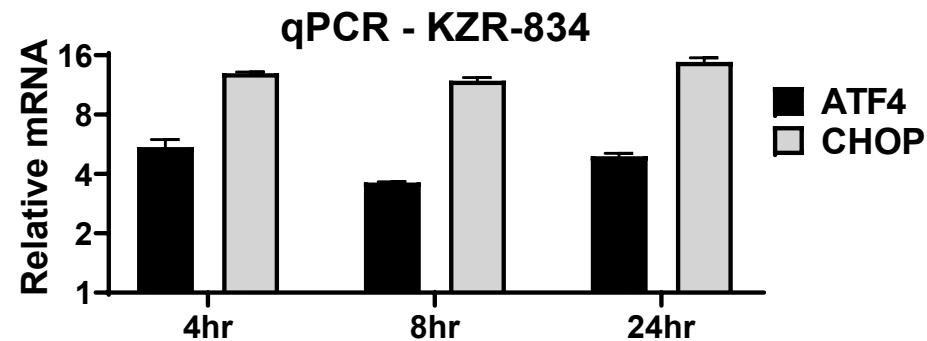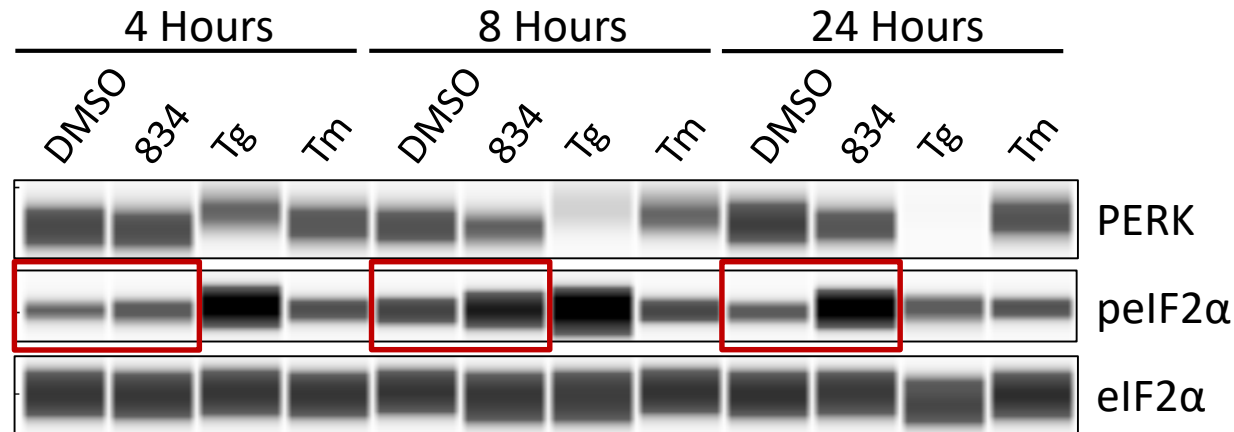834 (250nM); Tg - Thapsigargin (100nM); Tm - Tunicamycin (1ug/mL)

H929 - 834 + IRE1i (24hr)

IRE1 kinase inhibition by AMG18 partially protects against 834 cytotoxicity

Figure modified from Darling and Cook. *BBAMCR.* 2014; 1843 (10): 2150-2163

Activation of the Unfolded Protein Response: Prolonged Activation Downstream of PERK
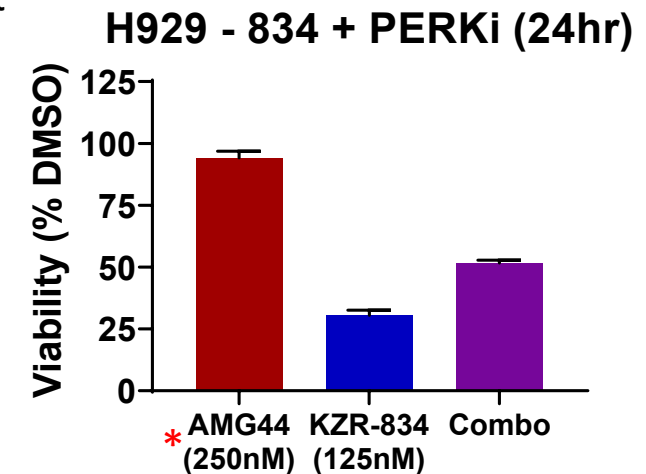
## PERK Branch of UPR

H929 (sensitive) Myeloma Cells

834 (250nM); Tg - Thapsigargin (100nM); Tm - Tunicamycin (1ug/mL)

qPCR - KZR-834

H929 - 834 + PERKi (24hr)

Modulation of PERK activity partially rescues 834 induced cell death

Sustained activation downstream of PERK through 24hr. Distinct profile from control ER stress inducers

Figure modified from Darling and Cook. *BBAMCR.* 2014; 1843 (10): 2150-2163

*Increased ATF4/CHOP mRNA observed at all concentrations of AMG44 tested alone and in combination with 834

Resistant U266 Cells Display Minimal UPR Compared to H929

U266 (resistant) Myeloma Cells

834 (250nM); Tg - Thapsigargin (100nM); Tm - Tunicamycin (1ug/mL)
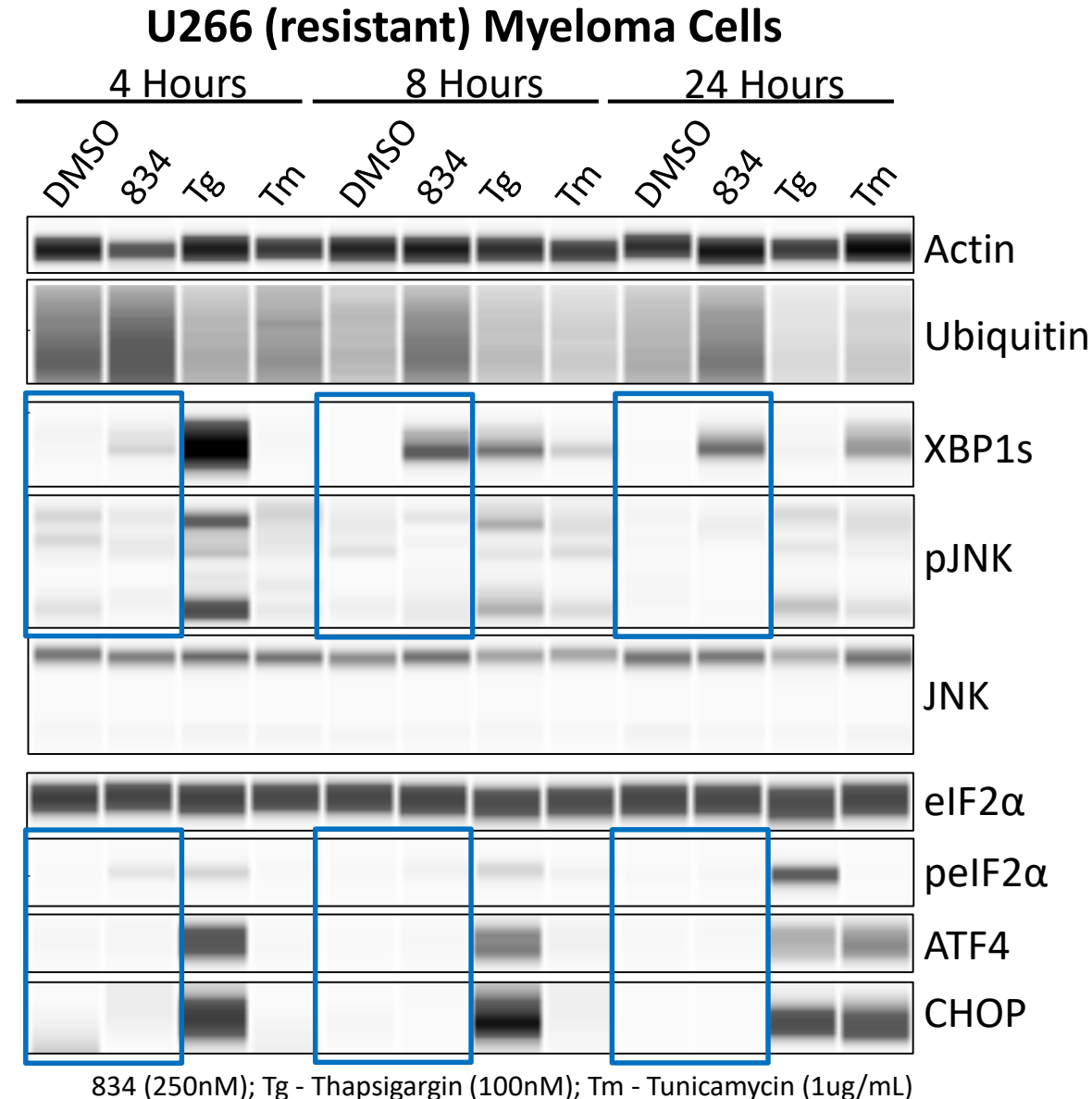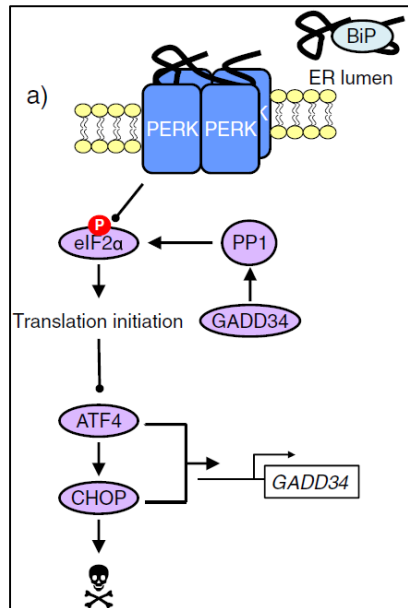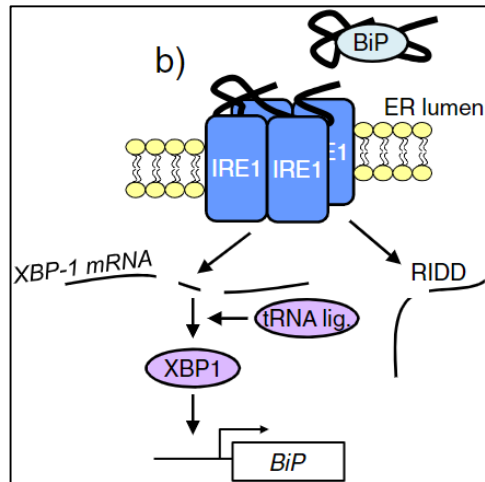
Minimal XBP1 splicing and JNK activation compared to H929

No pEIF2α, ATF4, or CHOP detected out to 24hr

Sec61 Inhibition Synergizes with Proteasome Inhibitor Carfilzomib

KZR-834 - 250nM (8hr)

H929 24hr Viability

- KZR-834 (78nM)
- CFZ (2nM)
- Combo

U266 24hr Viability

- KZR-834 (78nM)
- CFZ (3nM)
- Combo

H929

BLISS values		KZR-834 (nM)					
		1250	625	313	156	78	39
CFZ (nM)	3						
	2						
	1						

Synergy — Additive — Antagonism

U266

BLISS values		KZR-834 (nM)					
		1250	625	313	156	78	39
CFZ (nM)	3						
	2						
	1						
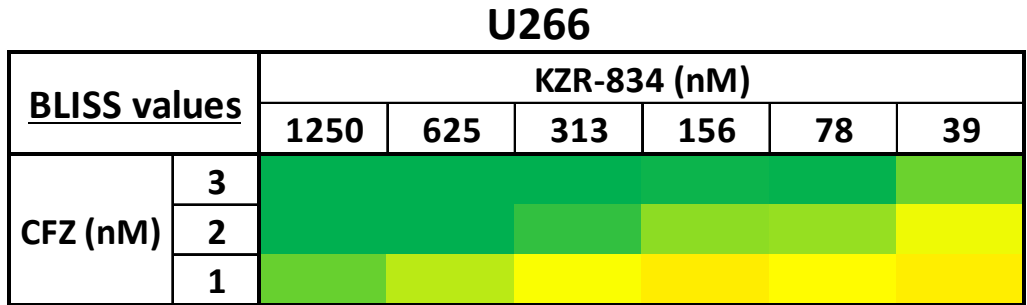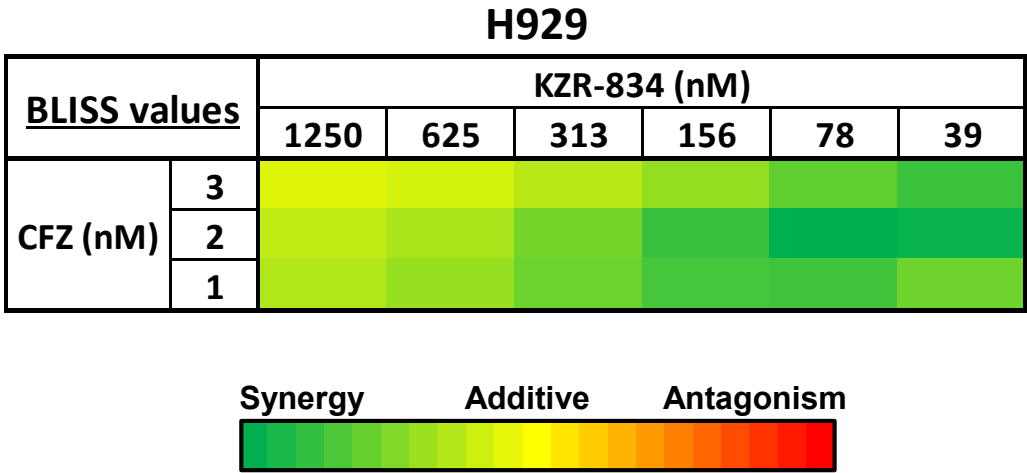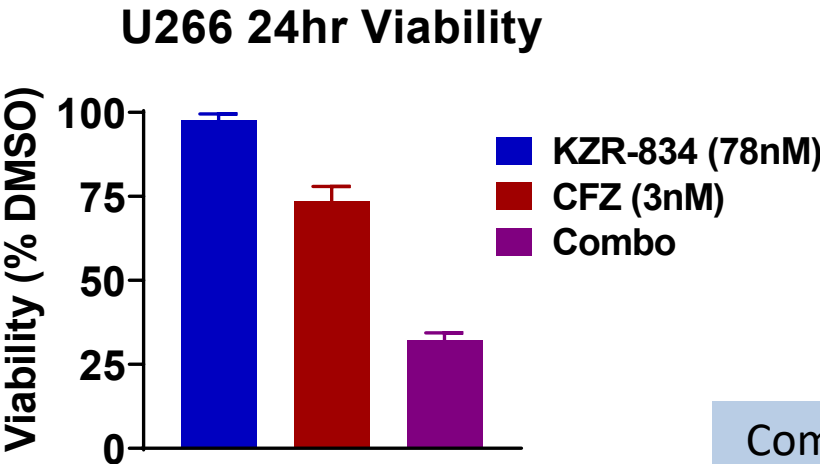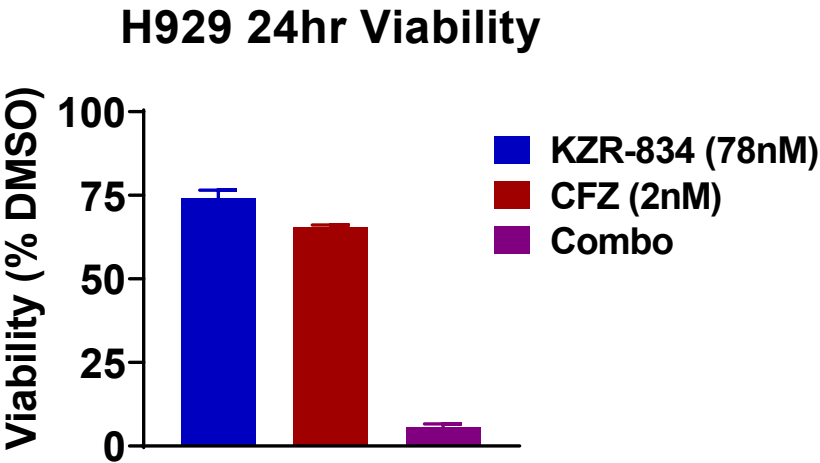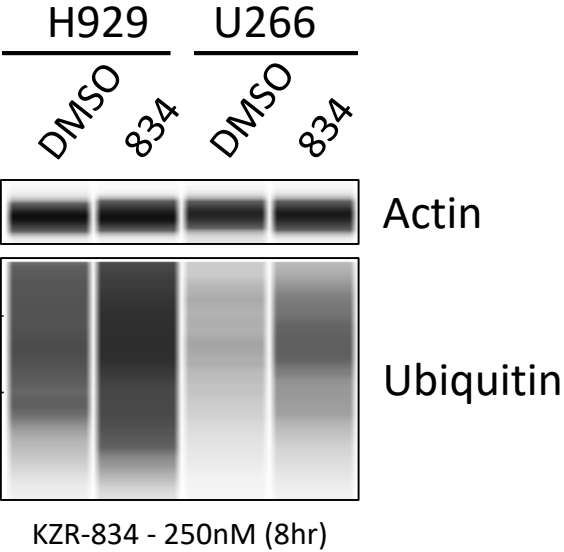
Combination with proteasome inhibitor synergizes in both sensitive and insensitive myeloma cells.

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High Basal Expression of ER Resident Proteins Provides Potential Predictor of Sensitivity
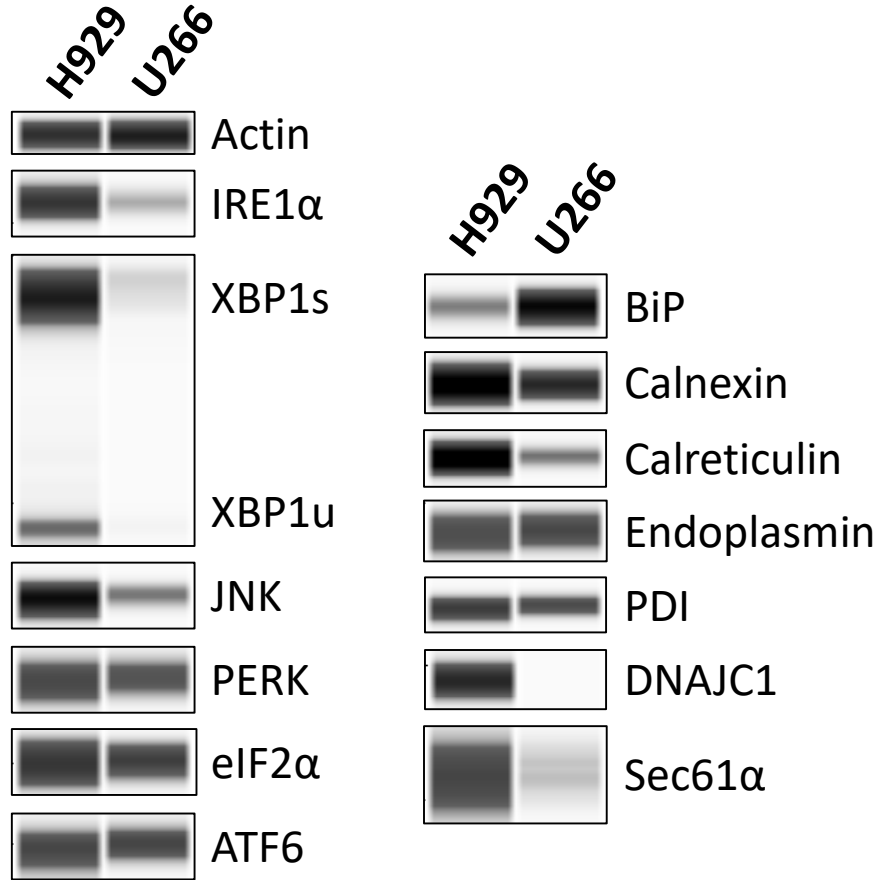
Increased Expression of ER Stress Response Genes (CCLE)
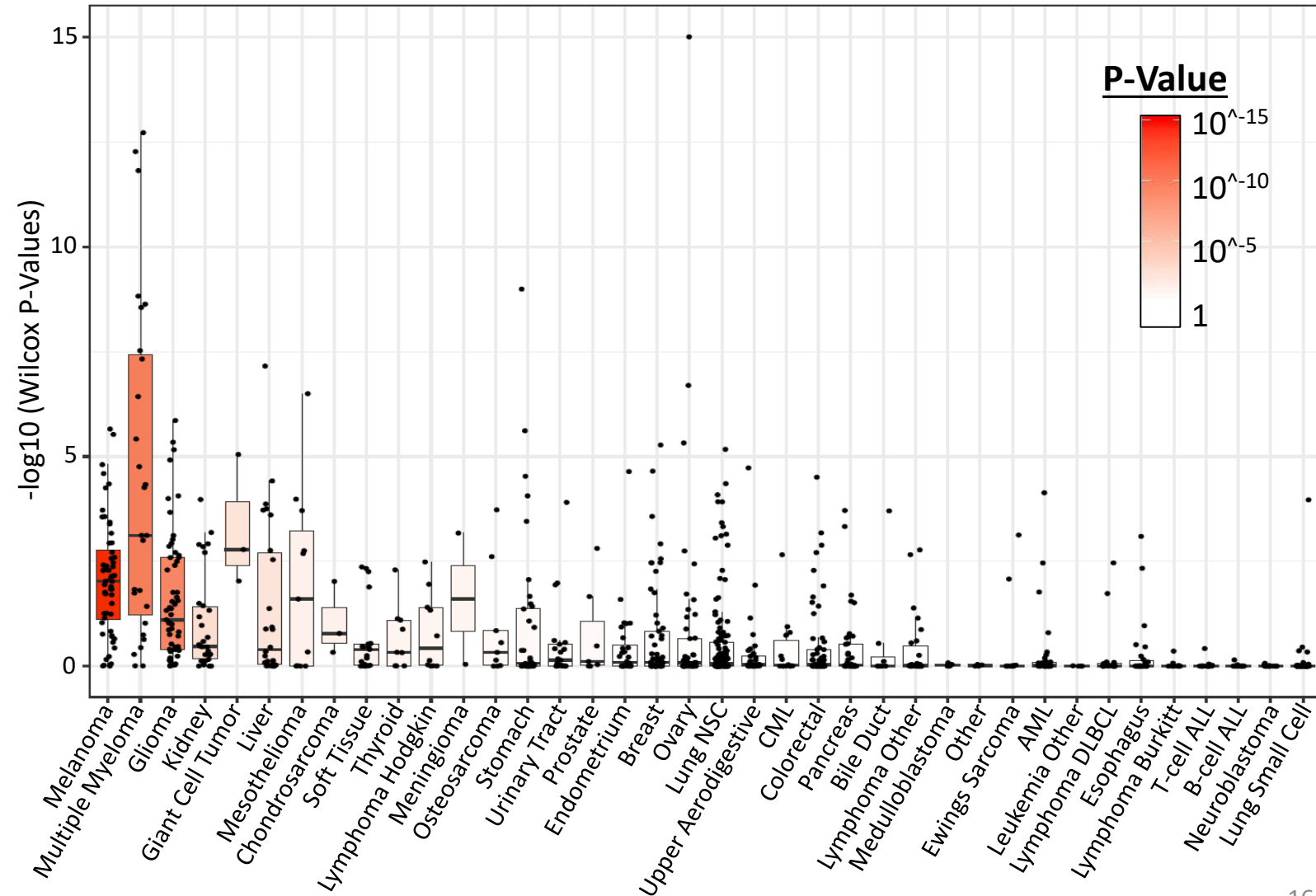
Summary

- Small molecule modulators of Sec61 can inhibit expression of multiple therapeutically relevant proteins including growth factors, cytokines, and immune checkpoints

- Sec61 inhibitors show broad anti-cancer activity against multiple hematologic tumor types in vitro and in vivo

- In sensitive myeloma cells, Sec61 blockade results in an unfolded protein response, providing a potential link between basal ER stress levels and sensitivity to Sec61 inhibition

- IND-enabling activities are underway for KZR-261

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