Supplementary Figures

Deletion of Cysteine Cathepsins B or L Yields Differential Impacts on Murine Skin Proteome and Degradome.

Tholen et al.

Supplementary Figure 1: Density graphs of fold change values ($\log_2$) for proteins identified in the biological replicates of the (A) Ctsb and (B) Ctsl experiment. A fold change of 0 indicates unaffected protein abundance. Fold change values lower than -0.58 and higher than 0.58 represent changes in protein abundance of more than 50 %.

Supplementary Figure 2: Volcano plots of the transcriptomic analysis of wild-type and (A) *Ctsb*$^{-/-}$ deficient MEFs or (B) *Ctsl*$^{-/-}$ deficient MEFs respectively. Gene expression was considered to be significantly up- or downregulated, if the averaged p-value is lower than 0.01. Red-marked genes are functionally annotated with the GO term "proteolysis" and genes marked in blue are functionally annotated with the GO term “regulation of proteolysis”. The Ctsl probe was not functional and yielded an insufficient signal in wild-type samples. However, Ctsl mRNA- and protein levels were independently validated by qPCR and immunoblotting thereby confirming wild-type and *Ctsl*$^{-/-}$ status.

Supplementary Figure 3: (A) CTSL degrades periostin at pH 5.5. Minor processing was observed at pH 7.0; (B) at neutral pH, minor processing of periostin was observed even in the presence of dextrane sulfate (10 ng/µl), which reportedly stabilizes cathepsin activity at neutral and basic pH (65). ON, overnight

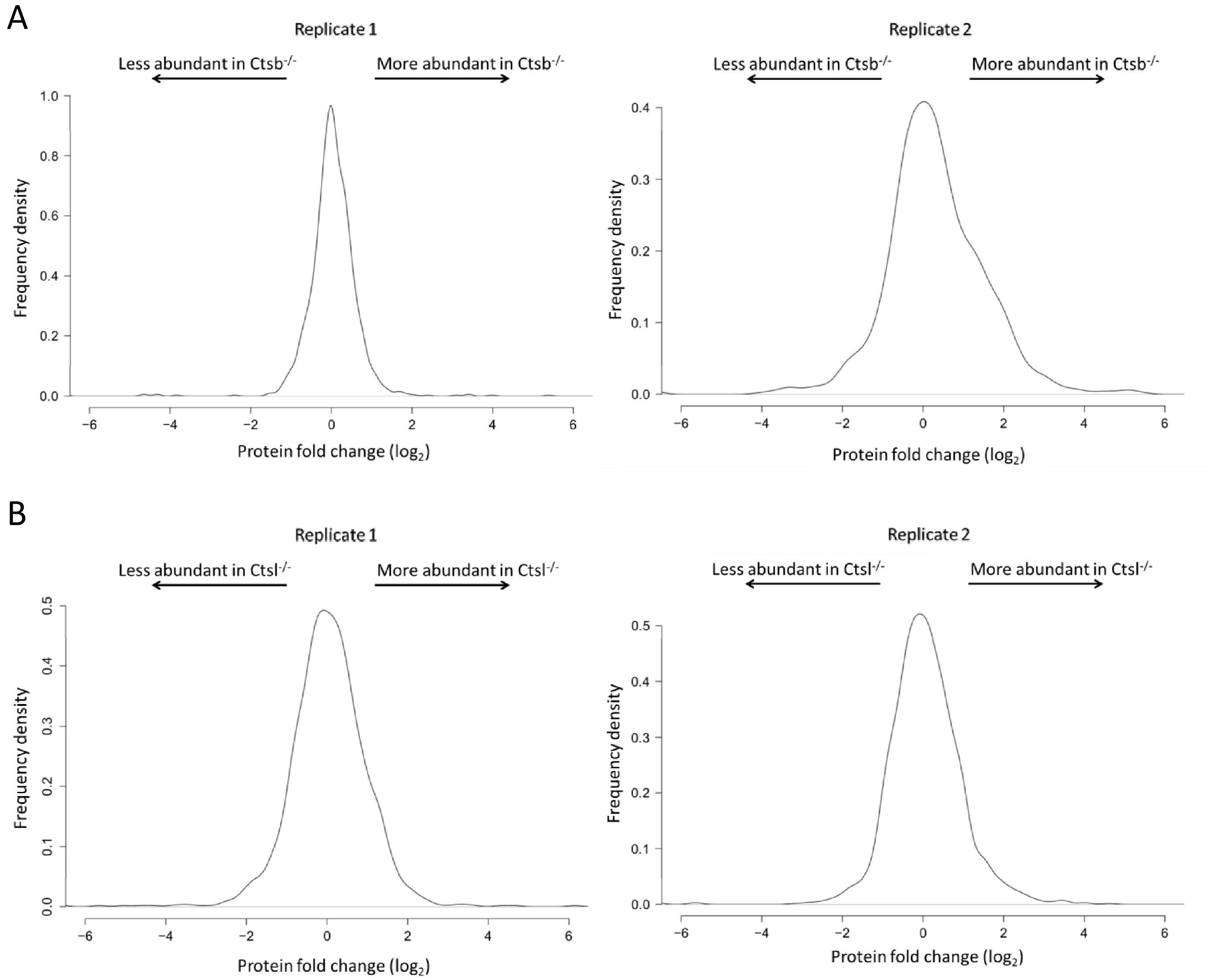
Supplementary Figure 4: (A/B) Pathways affected by Ctsb or Ctsl depletion and (C/D) protein classes affected by the depletion of Ctsb and Ctsl. Analysis was performed using the Panther Classification System (http://www.pantherdb.org) (66). Pathways or protein classes for which PANTHER determined an enrichment of proteins with increased abundance upon cathepsin depletion are labeled with “+”.Pathways or protein classes for which PANTHER determined an enrichment of proteins with decreased abundance upon cathepsin depletion are labeled with “-”.

Supplementary Figure 5: Cathepsin inhibition impairs *in vitro* angiogenesis. VEGF-induced vessel sprouting from rat aortic rings is reduced by the cysteine cathepsin inhibitor E64d. ** significant ($p<0.01$; ANOVA).

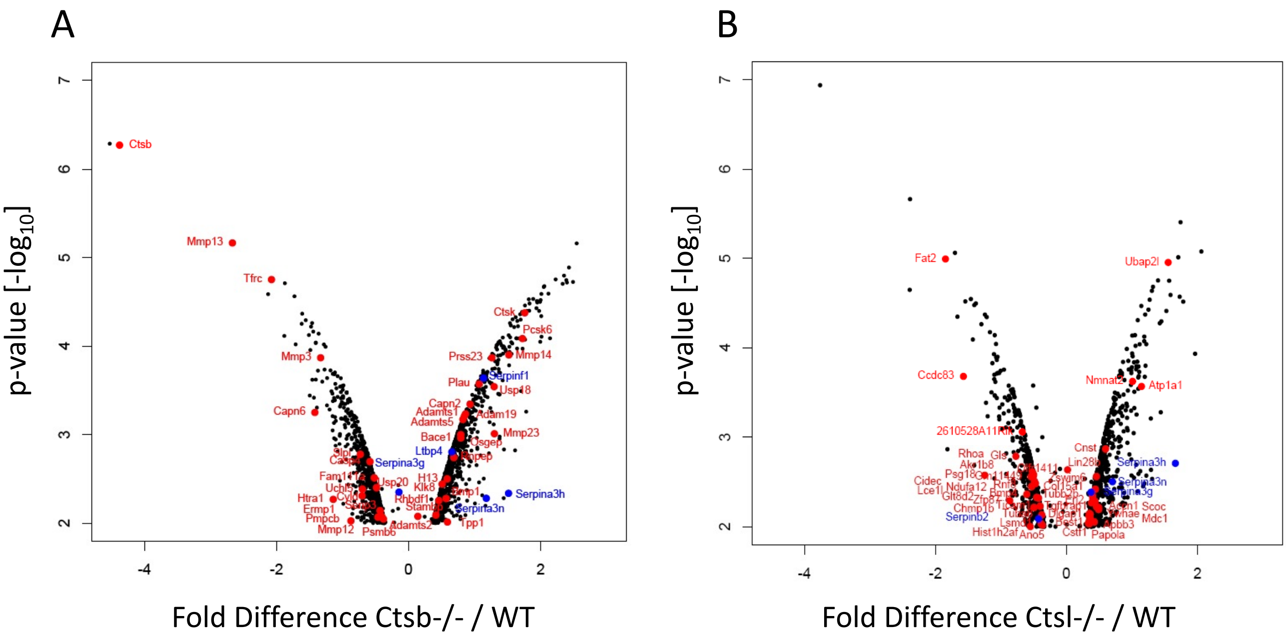
Supplementary Figure 6: Density graphs of peptide fold change values ($\log_2$) identified in the biological replicates of the (A) Ctsb and (B) Ctsl TAILS experiment. A fold change of 0 indicates unaffected peptide abundance. Fold change values lower than -0.58 and higher than 0.58 represent changes in peptide abundance of more than 50 %. Q_1: quantile 0 – 15; Q_2: quantile 15 – 25; Q_3: quantile 25 – 75; Q_4: quantile 75 – 85; Q_5: quantile 75 – 100

Supplementary Figure 7: Correlation graph of proteins identified in the proteome comparison for which the TAILS procedure identified an N-terminal peptide (analysis restricted to proteins and N-termini identified in both biological replicates).

Tholen *et al.*, Sup. Fig. S1



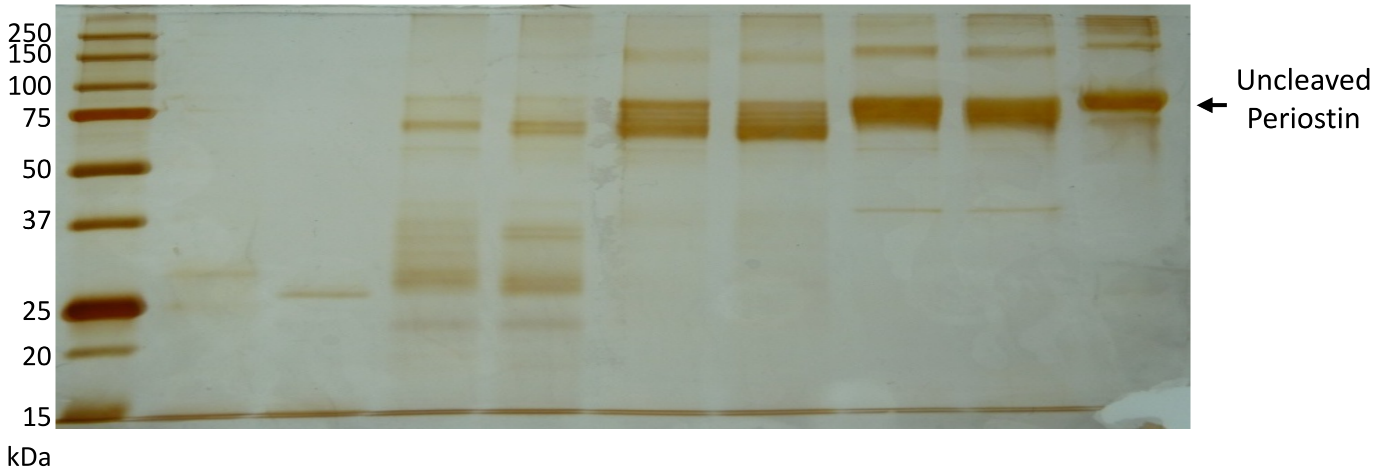
Tholen *et al.*, Sup. Fig. S2



Tholen *et al.*, Sup. Fig. S3

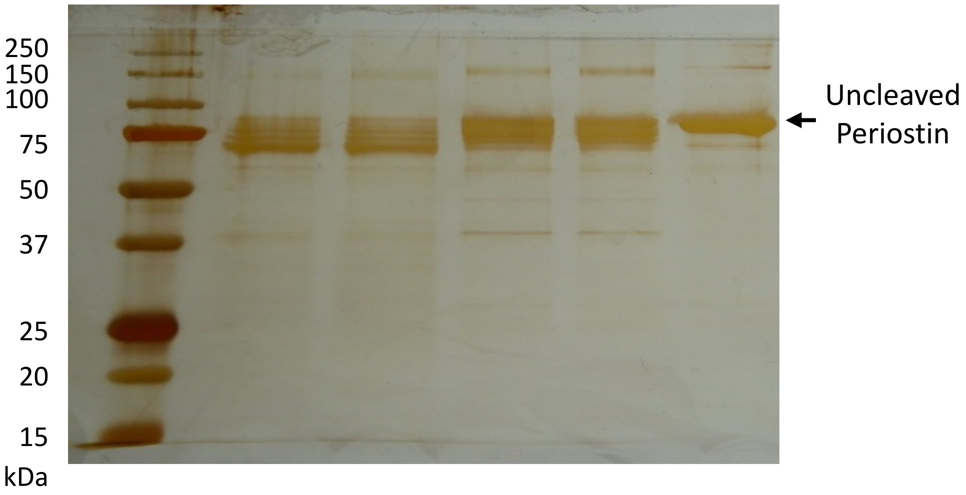
A

Periostin [µg]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
CTSL [µg]	0.2	0.2	0.02	0.02	0.2	0.2	0.02	0.02	-
Incubation	2h	ON	2h	ON	2h	ON	2h	ON	ON
pH	5.5	5.5	5.5	5.5	7.0	7.0	7.0	7.0	5.5



B

Periostin [µg]	2.0	2.0	2.0	2.0	2.0
CTSL [µg]	0.2	0.2	0.02	0.02	-
Incubation	2h	ON	2h	ON	ON
pH	7.0	7.0	7.0	7.0	7.0
Dextrane sulfate [ng/µl]	10	10	10	10	10



Tholen *et al.*, Sup. Fig. S4

A

WT/Ctsb$^{-/-}$			
Pathways	Number of proteins	+/-	p value
Ubiquitin proteasome pathway	28	+	6,48E-09
p53 pathway	11	+	4,41E-05
FGF signaling pathway	20	+	1,13E-04
Angiogenesis	18	+	3,60E-04
EGF receptor signaling pathway	21	+	7,52E-04
VEGF signaling pathway	10	+	2,38E-03
Parkinson disease	35	+	3,66E-03
PI3 kinase pathway	12	+	1,57E-02
PDGF signaling pathway	10	+	1,98E-02
Ras Pathway	12	+	2,92E-02
Blood coagulation	17	-	4,92E-02
TCA cycle	10	-	1,23E-01
Glycolysis	13	-	5,18E-01

B

WT/Ctsl$^{-/-}$			
Pathways	Number of proteins	+/-	p value
Ubiquitin proteasome pathway	30	+	2,46E-05
Blood coagulation	17	-	2,67E-05
TCA cycle	10	-	6,32E-04
p53 pathway	12	+	1,49E-03
FGF signaling pathway	19	+	3,56E-03
Parkinson disease	34	+	6,12E-03
PI3 kinase pathway	11	+	6,25E-03
Glycolysis	16	-	1,43E-02
EGF receptor signaling pathway	21	+	3,29E-02
Ras Pathway	13	+	2,44E-01
PDGF signaling pathway	10	+	2,75E-01
VEGF signaling pathway	10	-	7,51E-01
Angiogenesis	19	+	8,03E+01

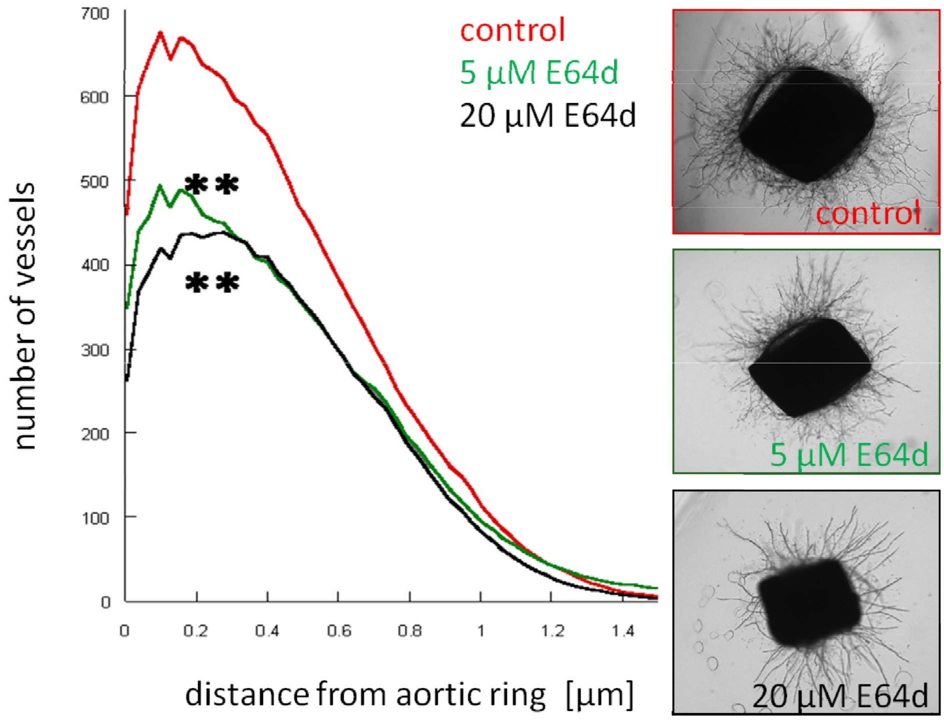
C

WT/Ctsb$^{-/-}$			
Protein Class	Number of proteins	+/-	p value
oxidoreductase	107	-	5,46E-07
chaperone	32	+	9,82E-06
dehydrogenase	64	-	2,90E-04
nucleic acid binding	62	+	2,34E-03
metalloprotease	18	-	2,84E-03
serine protease inhibitor	21	-	4,10E-03
ligase	29	+	1,01E-02
protease inhibitor	23	-	1,03E-02
DNA binding protein	13	+	1,30E-02
RNA binding protein	43	+	1,32E-02
isomerase	30	-	1,46E-02
receptor	36	-	2,81E-02
structural protein	10	+	2,94E-02
ATP synthase	10	-	4,54E-02

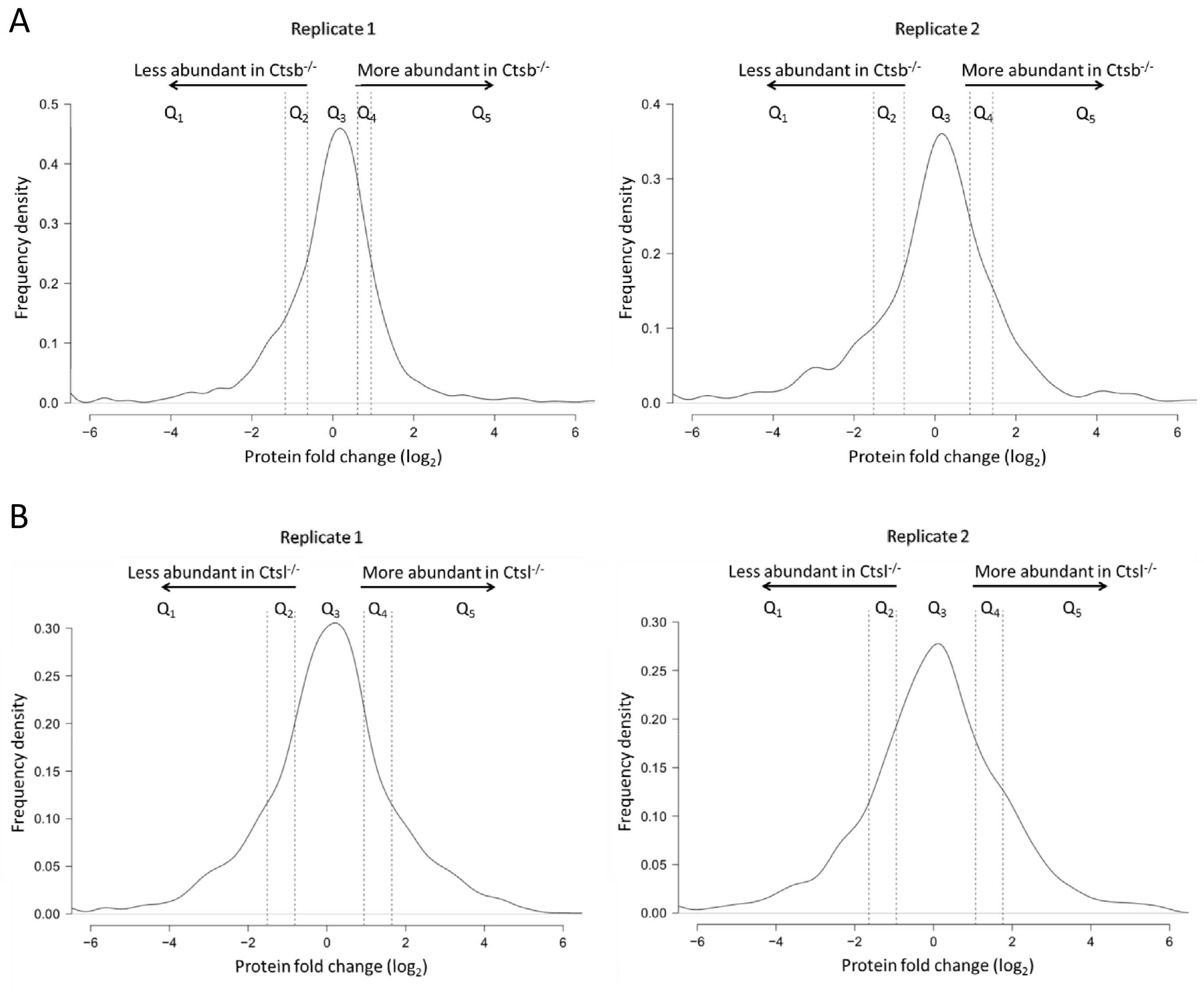
D

WT/Ctsl$^{-/-}$			
Protein Class	Number of proteins	+/-	p value
nucleic acid binding	70	+	2,43E-06
chaperone	32	+	3,14E-06
serine protease inhibitor	23	-	8,03E-06
protease inhibitor	27	-	2,87E-05
oxidoreductase	113	-	3,00E-05
dehydrogenase	64	-	1,06E-04
RNA binding protein	47	+	1,93E-04
mRNA processing factor	13	+	5,98E-04
DNA binding protein	17	+	6,25E-04
cysteine protease	23	+	3,41E-03
ligase	35	+	9,61E-03
transferase	150	-	2,25E-02
lyase	21	-	3,94E-02
microtubule family cytoskeletel p	17	+	4,43E-02

Tholen *et al.*, Sup. Fig. S5



Tholen *et al.*, Sup. Fig. S6



Tholen *et al.*, Sup. Fig. S7