Supplemental Materials

Molecular Biology of the Cell

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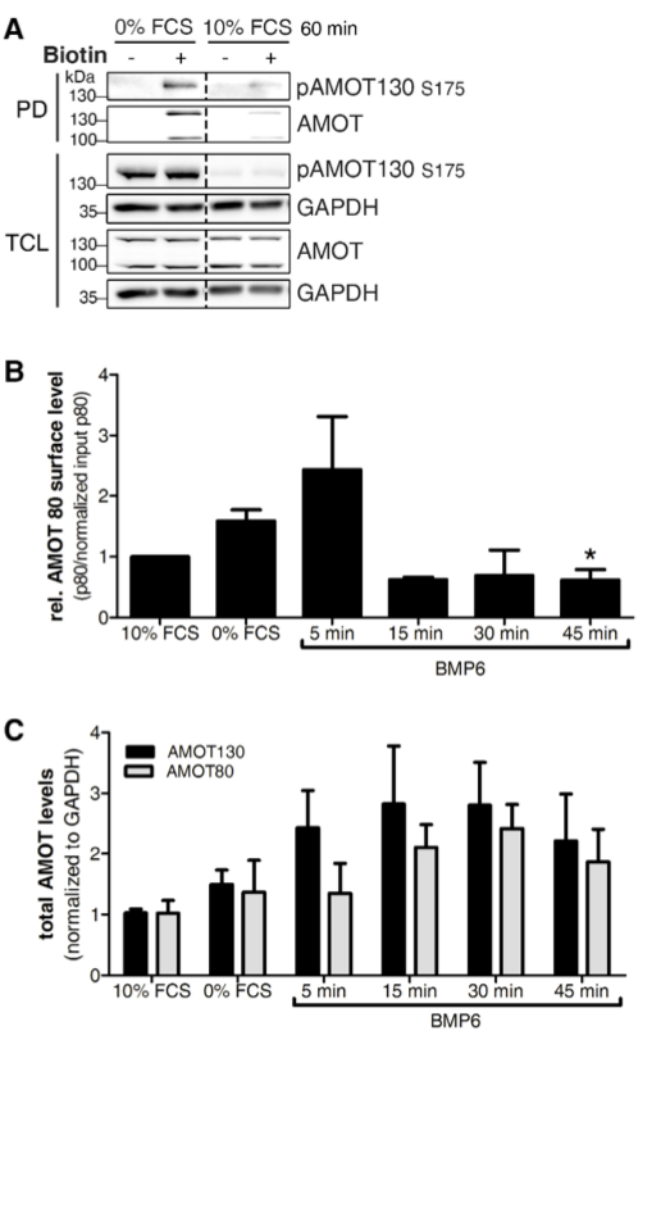
Supplementary Figure Legends

Supplemental Figure 1: (A) HEK293T cells were starved with 0% serum and stimulated with 10% serum, subjected to surface biotinylation, lysed and biotinylated proteins were precipitated with Streptavidin beads. Eluted proteins were immunoblotted using indicated antibodies. Pulldown (PD) with Streptavidin beads only represents proteins expressing an extracellular motif. Total cell lysate (TCL) is the protein input before Streptavidin pulldown. (B) AMOT80 surface level expression is also decreased after stimulation with BMP6 for 45 min. Densitometric analysis of blots depicted in Figure 1A. (C) Total AMOT levels do not differ between conditions. Densitometric analysis of blots depicted in Figure 1A. Supplemental Videos of Live Cell Imaging in MCF7 cells. Video "SVideo4_starve.mov" depicts starving control and video "SVideo5_BMP6.mov" presents BMP6 stimulated cell.

Supplemental Figure 2: AMOT130 but not AMOT80 dynamically associates to the BMPR2 and SMAD1. (A-C) Transfected HEK293T cells were subjected to immunoprecipitation using either α-AMOT (TLE) (A) or α-HA tag (B, C) antibody. Before, cells were left in full medium. Immunoprecipitates (IP) and TCL were analyzed by Western blotting using indicated antibodies. Incubation with rabbit (A) or mouse IgG (B, C) served as control. (D) *In situ* proximity ligation assay (PLA) controls of experiment depicted in Figure 2. MCF7 cells were subjected to *in situ* PLA (green signal). Nuclei were stained with DAPI (blue) and F-actin with Phalloidin594 (red). PLA signal images were inverted to visualize the signal. Scale bar represents 20 µm. AMOT-YAP association served as positive control. AMOT or SMAD1 only antibody served as negative control.

Supplemental Figure 3: Targeted AMOT depletion specifically reduces phosphorylation of BMP-SMAD proteins. (A-C) Mesenchymal precursor cells were transfected with siRNA targeting either nonspecific sequences (si-scr) or human/mouse AMOT (si-*AMOT*) and stimulated for 30 min with 10 nM BMP2. Protein lysates of respective cell lines, C2C12 (A), human myoblasts (B), hFOBs (C) were subjected to Western blotting using indicated antibodies. Blots represent at least three independent experiments. (D-H) AMOT knockdown was validated on protein (D-G) and RNA level in MCF7 cells (H). Quantification depicts efficient knockdown of more than 80% AMOT protein (D). (I, J) Target gene expression of MCF7 cells measured by qPCR after 1 h of BMP6 stimulation in transwell experiments. After 1 h of stimulation, MCF7 cells were lysed and RNA was extracted, reverse transcribed and used for gene expression analysis of *ID2* and *ID3* mRNA. Data of qPCR analyses is presented as mean Fold Induction (F.I.) ± SEM of four independent experiments. *** $p < 0.001$, two-way ANOVA with Bonferroni post-hoc test, compared to respective si-scr control.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0% FCS
10% FCS
60 min
Biotin
-
+
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kDa
PD
130
100
pAMOT130 S175
AMOT
TCL
130
35
pAMOT130 S175
GAPDH
130
100
AMOT
35
GAPDH
B
rel. AMOT 80 surface level
(p80/normalized input p80)
4
3
2
1
0
*
10% FCS
0% FCS
5 min
15 min
30 min
45 min
BMP6
C
total AMOT levels
(normalized to GAPDH)
AMOT130
AMOT80
4
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10% FCS
0% FCS
5 min
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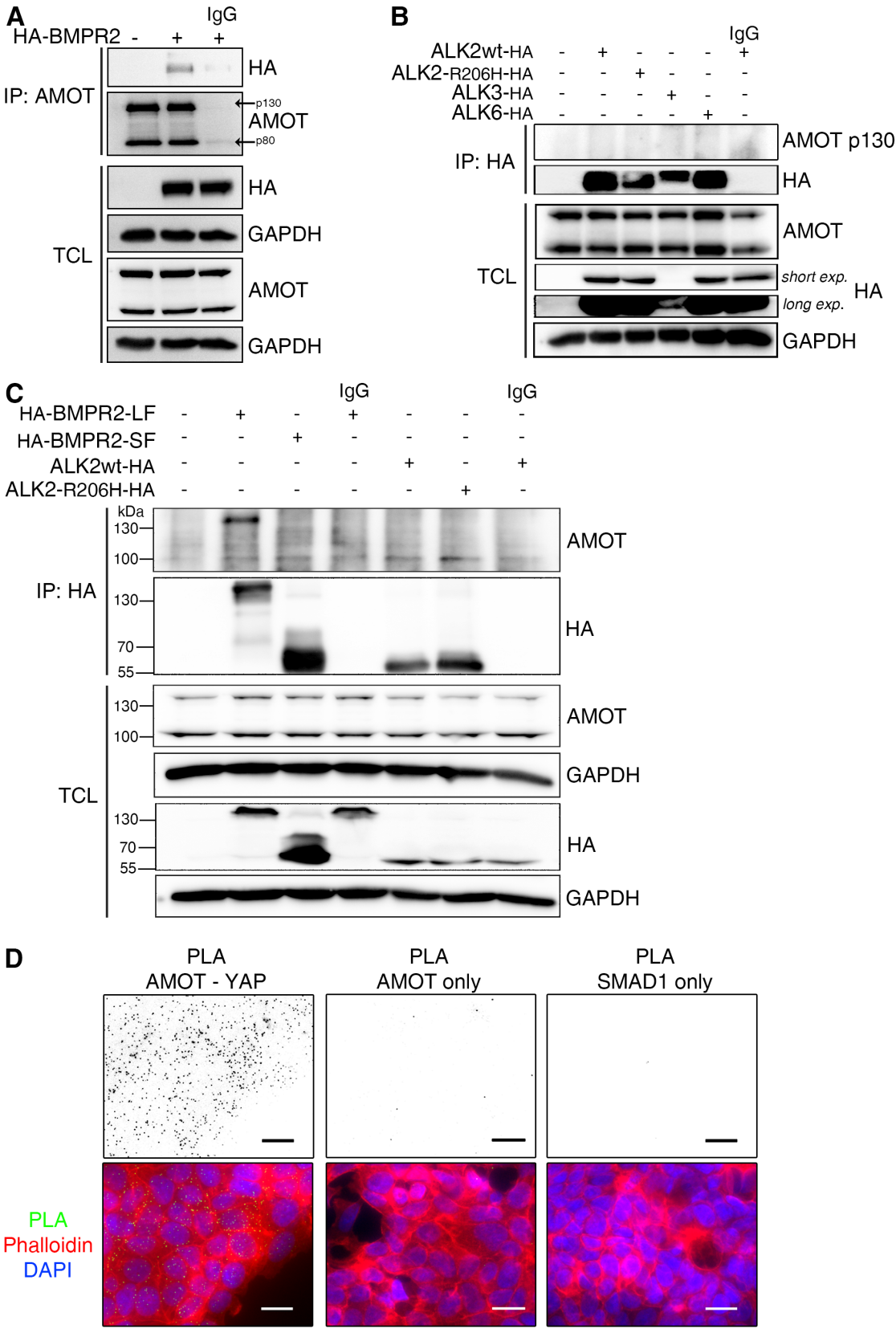
30 min
45 min
BMP6


A
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IP: AMOT
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AMOT
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B
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ALK2wt-HA - + - - - +
ALK2-R206H-HA - - + - - -
ALK3-HA - - - + - -
ALK6-HA - - - - + -
IP: HA
AMOT p130
HA
TCL
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short exp.
long exp.
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GAPDH
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HA-BMPR2-SF - - + - - - -
ALK2wt-HA - - - - + - +
ALK2-R206H-HA - - - - - + -
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70
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HA
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GAPDH
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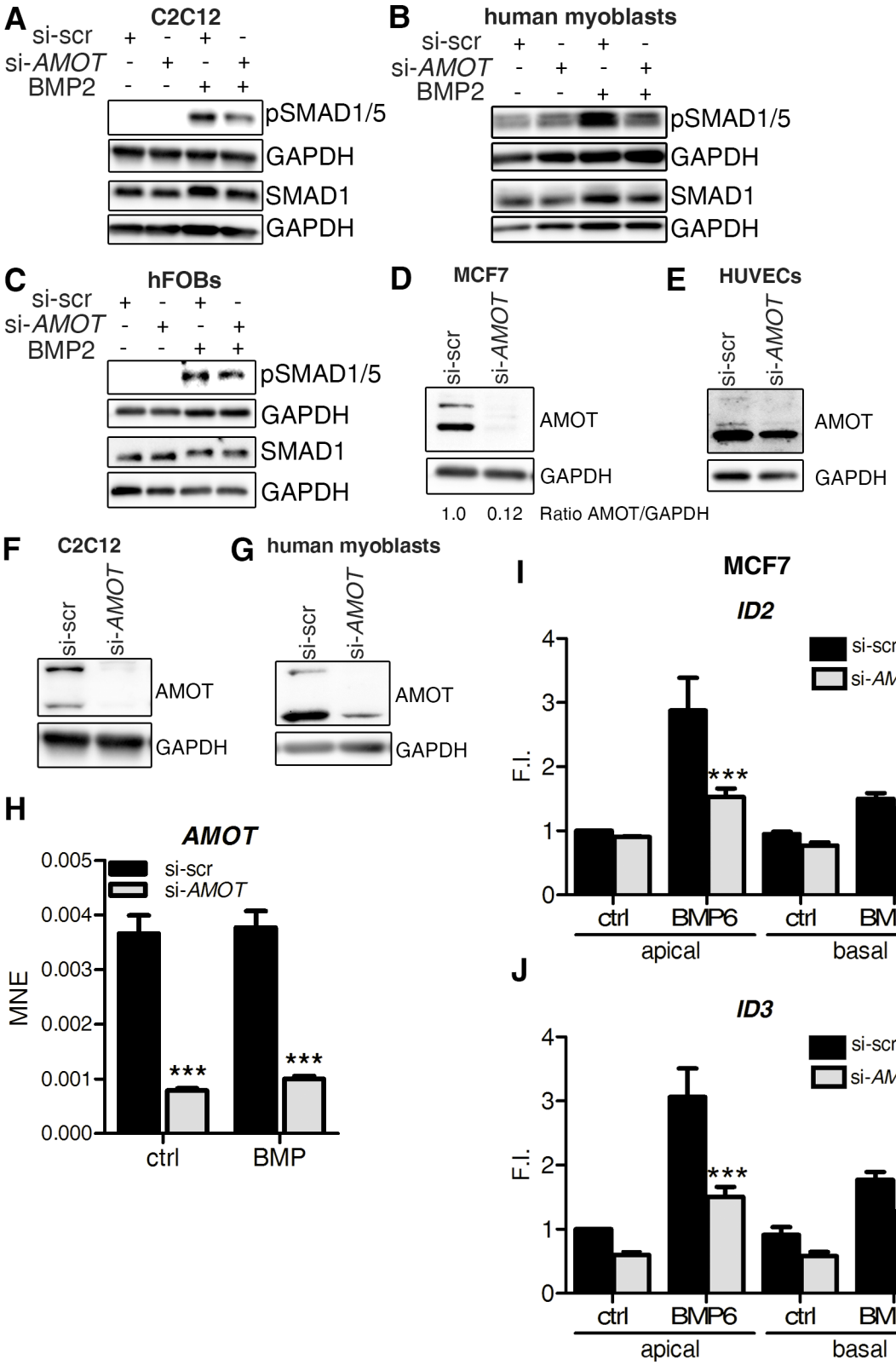
AMOT - YAP
PLA
AMOT only
PLA
SMAD1 only
PLA
Phalloidin
DAPI


A
C2C12
si-scr + - + -
si-AMOT - + - +
BMP2 - - + +
pSMAD1/5
GAPDH
SMAD1
GAPDH
B
human myoblasts
si-scr + - + -
si-AMOT - + - +
BMP2 - - + +
pSMAD1/5
GAPDH
SMAD1
GAPDH
C
hFOBs
si-scr + - + -
si-AMOT - + - +
BMP2 - - + +
pSMAD1/5
GAPDH
SMAD1
GAPDH
D
MCF7
si-scr
si-AMOT
AMOT
GAPDH
1.0 0.12 Ratio AMOT/GAPDH
E
HUVECs
si-scr
si-AMOT
AMOT
GAPDH
F
C2C12
si-scr
si-AMOT
AMOT
GAPDH
G
human myoblasts
si-scr
si-AMOT
AMOT
GAPDH
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AMOT
si-scr
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MNE
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si-scr
si-AMOT
F.I.
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2
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n.s.
ctrl
BMP6
ctrl
BMP6
apical
basal

Video legends

SVideo 1: Video depicts a z-stack of MCF7 cells subjected to immunofluorescence staining of AMOT (green) and PKCzeta (red). Nuclei are stained with DAPI (blue). Z-stack is shown from basal to apical. For representative picture see Figure 5.

SVideo 2: Video depicts a z-stack of MCF7 cells subjected to immunofluorescence staining of ZO-1 (green) and PKCzeta (red). Nuclei are stained with DAPI (blue). Z-stack is shown from basal to apical. For representative picture see Figure 5.

SVideo 3: Video depicts a z-stack of MCF7 cells subjected to immunofluorescence staining of BMPR2 (green) and PKCzeta (red). Nuclei are stained with DAPI (blue). Z-stack is shown from apical to basal. For representative picture see Figure 5.

SVideo 4_starve: Video depicts a representative MCF7 cell, expressing GFP tagged AMOT130 and incubated in a live cell incubation chamber. Cells were starved and images of the GFP signal were taken every 30 seconds for 1 h. For representative pictures and quantification see Figure 1.

SVideo 5_BMP6: Video depicts a representative MCF7 cell, expressing GFP tagged AMOT130 and incubated in a live cell incubation chamber. Cells were starved and then stimulated with BMP6 for 1 h. images of the GFP signal were taken every 30 seconds for 1 h. For representative pictures and quantification see Figure 1.