| dox	-	+	
TGF-ß1	+	+	kDa
TGFBR2			65-75
pSMAD2			60
SMAD2			60

Supplementary Figure S1. Doxycycline-induced reconstitution of TGFBR2 expression and downstream signaling. Western blot analysis demonstrated dox-induced reconstituted expression of TGFBR2 and phosphorylation of SMAD2 (pSMAD2) in protein lysates of HCT116-TGFBR2 cells. Total SMAD2 protein was used as a loading control. Protein sizes are indicated. .
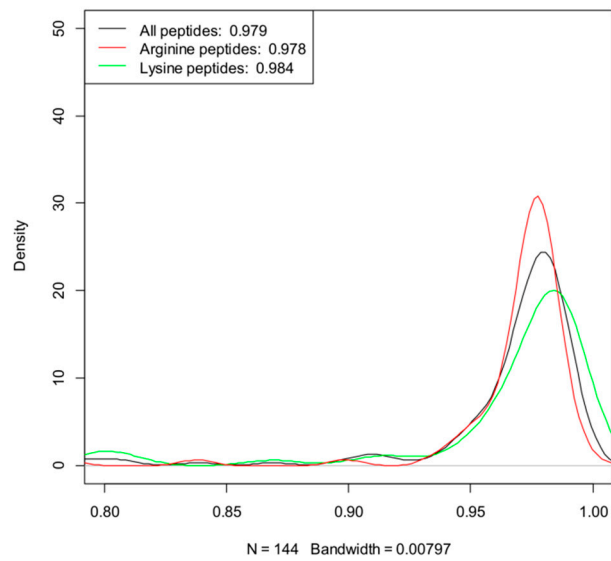
Supplementary Figure S2 Incorporation of heavy-labeled amino acids in EVs isolated from TGFBR2-proficient HCT116-TGFBR2 cells. Incorporation efficiency of heavy-labeled amino acids was calculated based on 144 identified peptides.
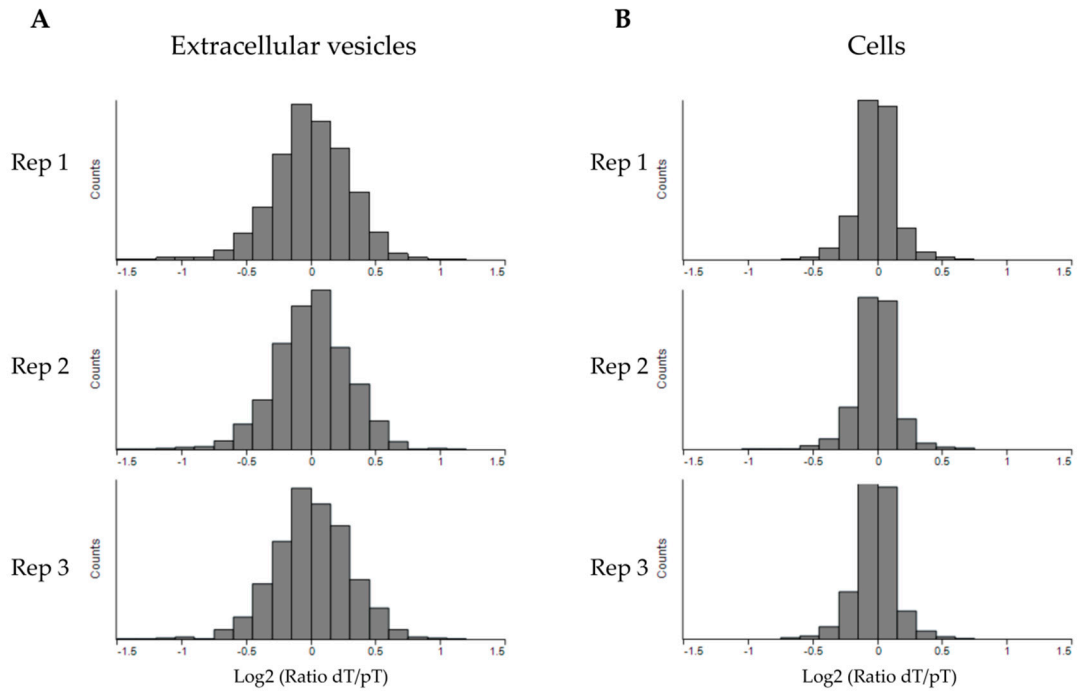
Supplementary Figure S3. Distribution of quantified protein ratios. Histograms are shown for $\log_2$-transformed protein ratios (dT/pT) from each biological replicate of EVs (A) and cells (B).

Supplementary Table S1 TGFBR2-regulated proteins in cells.

Gene names	Protein names	Protein ratio [1] (dT/pT)	Peptides (unique)	Coverage (%)	Identification score
Upregulated cellular proteins by TGFBR2 deficiency					
NUMA1	Nuclear mitotic apparatus protein 1	2.23	12 (12)	8.3	36.38
CAD	CAD protein	1.91	20 (20)	13.1	85.112
GLUL	Glutamine synthetase	1.82	7 (7)	25.5	29.43
UBE2Z	Ubiquitin-conjugating enzyme E2 Z	1.77	4 (4)	13.3	13.23
TUBB4A	Tubulin beta-4A chain	1.71	17 (17)	6.3	26.76
EDC4	Enhancer of mRNA-decapping protein 4	1.56	7 (7)	9.0	27.22
RANBP2	E3 SUMO-protein ligase RanBP2	1.51	6 (6)	3.4	20.66
Downregulated cellular proteins by TGFBR2 deficiency					
MGST3	Microsomal glutathione S-transferase 3	0.61	3 (3)	32.2	15.32

KRT18	Keratin, type I cytoskeletal 18	0.61	30 (19)	77	323.31
VDAC3	Voltage-dependent anion-selective channel protein 3	0.63	9 (9)	41.7	63.09
EMG1	Ribosomal RNA small subunit methyltransferase NEP1	0.64	4 (4)	22.1	15.02
KRT8	Keratin, type II cytoskeletal 8	0.65	39 (31)	64.2	323.31
VDAC2	Voltage-dependent anion-selective channel protein 2	0.66	13 (13)	59.5	174.22
HN1	Hematological and neurological expressed 1 protein	0.67	3 (3)	54.5	18.21

[1] Mean ratios calculated from three biological replicates.