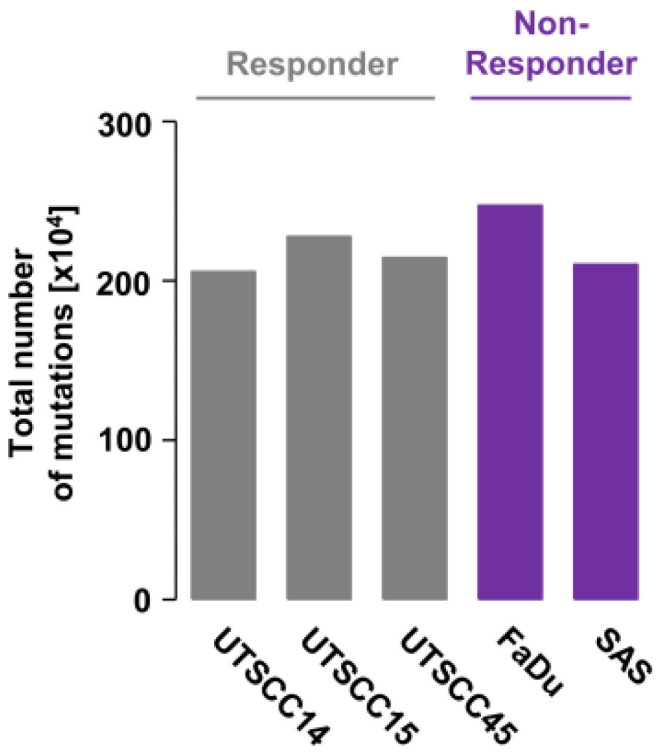
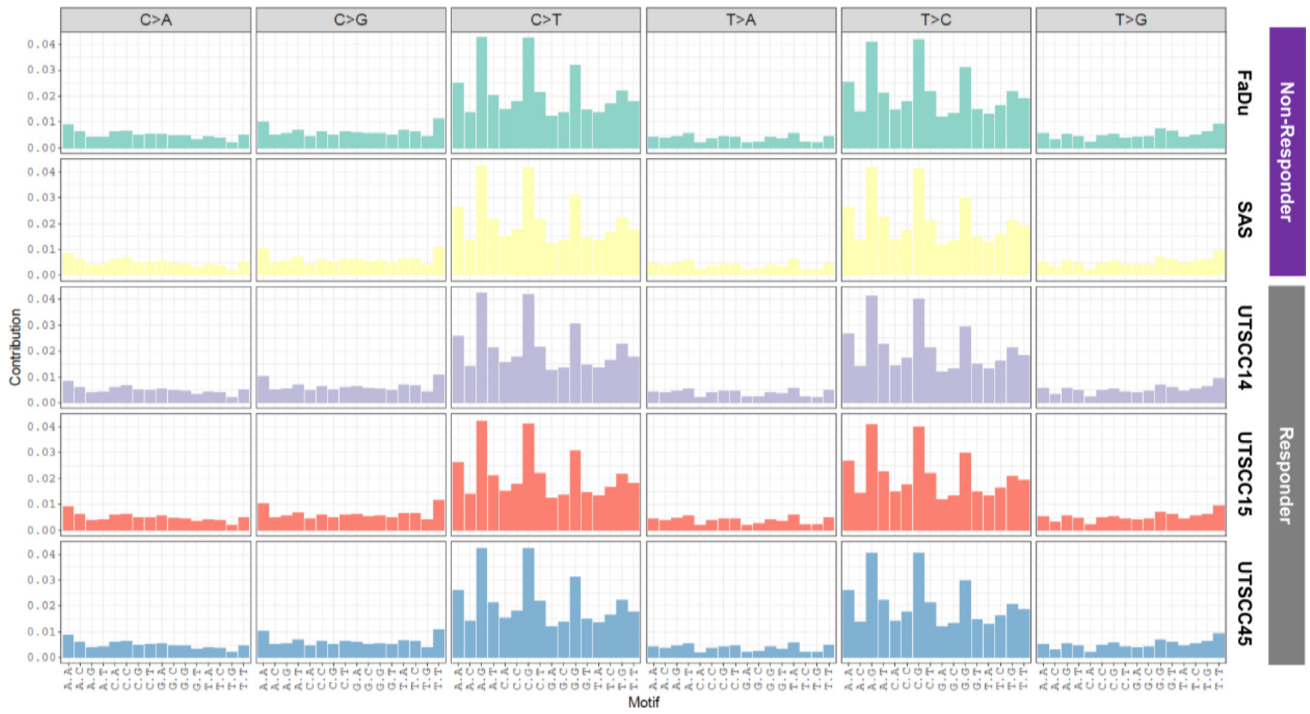
Whole exome sequencing identifies mTOR and KEAP1 as potential targets for radiosensitization of HNSCC cells refractory to EGFR and β1 integrin inhibition

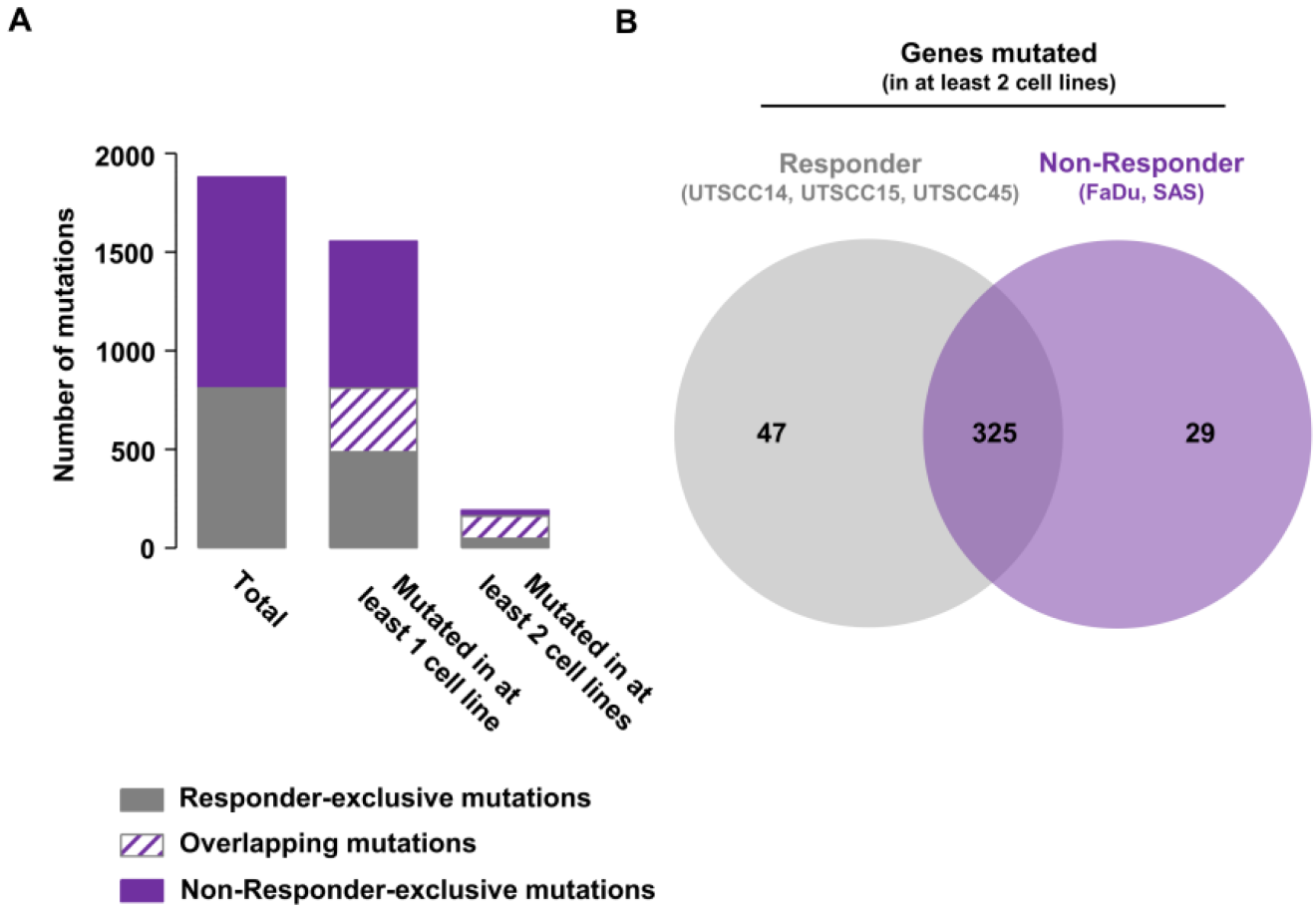
SUPPLEMENTARY MATERIALS



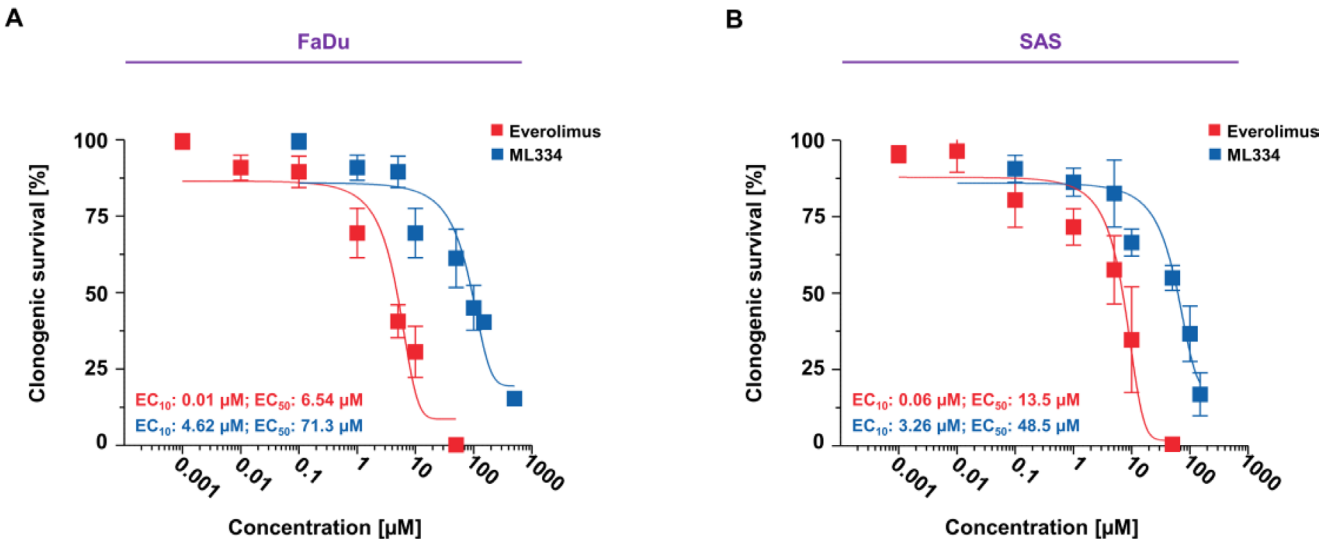
Supplementary Figure 1: Mutations found in responder and non-responder cells. Total number of non-coding and coding mutations detected in 3D grown responder (grey) and non-responder (purple) HNSCC cell lines.



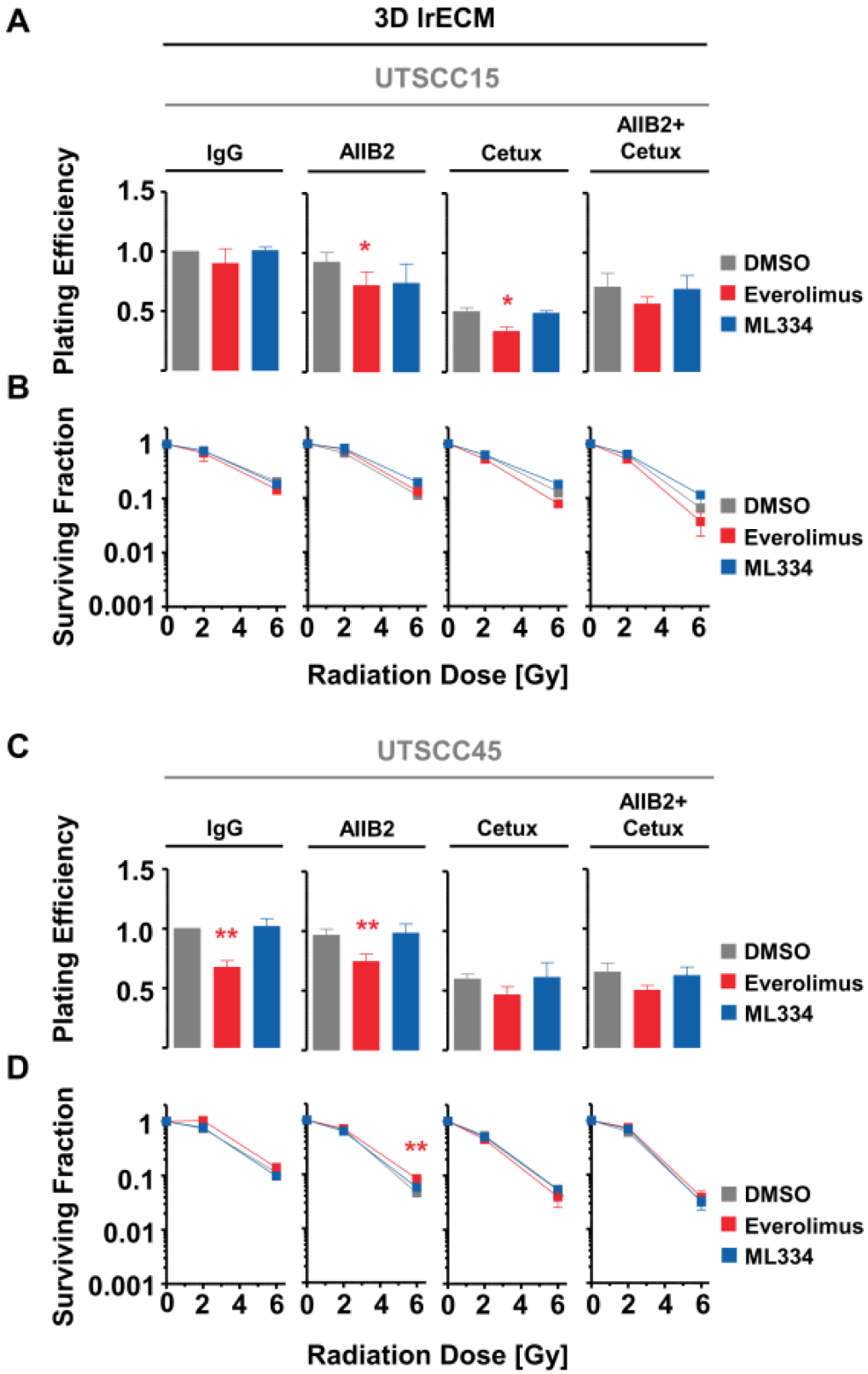
Supplementary Figure 2: Mutational motifs of responder and non-responder cells. Graphical representation of signatures identified in 3D lrECM-based UTSCC14, UTSCC15 and UTSCC45 (responders) as well as FaDu and SAS (non-responders) cell cultures. Mutations were identified by whole-exome sequencing.



Supplementary Figure 3: Mutational profiles of responder and non-responder cells. (**A**) Number of coding mutations detected in 3D grown responder (grey) and non-responder (purple) HNSCC cell lines. (**B**) Venn diagram analysis of all mutated genes showing shared mutations and exclusively mutated genes in the responder or non-responder groups.



Supplementary Figure 4: Cytotoxicity of Everolimus and ML334. (**A and B**) Everolimus (mTORi) and ML334 (KEAP1i) cytotoxicity and corresponding EC10 and EC50 values in FaDu (A) and SAS (B) cells (colony formation assay). Results show mean ± SEM ($n = 3$).



Supplementary Figure 5: Pharmacological inhibition of KEAP1 and mTOR fails to further radiosensitize AIIB2/Cetuximab treated UTSCC15 and UTSCC45 responder cells. (**A**) Cytotoxicity measured as clonogenic survival and (**B**) clonogenic radiation survival of UTSCC15 cells upon treatment with 10 nM Everolimus (mTOR inhibitor) or 3.3 μM ML334 (KEAP1i) simultaneously to AIIB2, Cetuximab or AIIB2/Cetuximab plus 2–6 Gy X-rays (DMSO, IgG and 0 Gy as control). (**C**) Cytotoxicity measured as clonogenic survival and (**D**) clonogenic radiation survival of UTSCC45 cells upon treatment with 10 nM Everolimus (mTOR inhibitor) or 3.3 μM ML334 (KEAP1i) simultaneously to AIIB2, Cetuximab or AIIB2/Cetuximab plus 2–6 Gy X-rays (DMSO, IgG and 0 Gy as control).

Supplementary Table 1: Number and type of mutations detected in HNSCC responder (UTSCC14, UTSCC15, UTSCC45) and non-responder (FaDu, SAS) cells using whole exome sequencing

Type	UT14	UT15	UT45	FaDu	SAS
A>C	5750	6429	5951	7007	5950
A>G	21434	24778	22636	27695	21975
A>T	4227	4744	4416	5108	4388
C>A	5307	5882	5487	6696	5423
C>G	6627	7395	7027	8445	6664
C>T	21336	24569	22746	27563	22017
G>A	21383	24398	22730	27672	21921
G>C	6580	7385	6843	8376	6572
G>T	5224	5984	5451	6777	5396
T>A	4367	4841	4518	5184	4353
T>C	21686	24972	22580	27760	22247
T>G	5620	6448	5900	7037	5676
Insertion	11147	13135	11830	14858	11758
Deletion	13702	16251	14728	18766	14460
SNV	129541	147825	136285	165320	132582
MNV	3153	3514	3459	3685	3360
Replacement	527	592	551	680	574
Total	158070	181317	166853	203309	162734

Supplementary Table 2: ID, gene name and sequence of used esiRNAs. See Supplementary_Table_2

Supplementary Table 3: Results of the esiRNA screen in 3D grown FaDu cells for the indicated treatments. *P*-values are referring to the corresponding RLUC controls. See Supplementary_Table_3

Supplementary Table 4: Results of the esiRNA screen in 3D grown SAS cells for the indicated treatments. *P*-values are referring to the corresponding RLUC controls. See Supplementary_Table_4

Supplementary Table 5: Data of the ratio of phosphorylated protein to non-phosphorylated protein levels from phosphoproteome array of 3D cultured UTSCC14, UTSCC15, UTSCC45, SAS and FaDu cells. See Supplementary_Table_5