Supplementary materials for

Agonist-specific protein interactomes of glucocorticoid and androgen receptor as revealed by proximity mapping

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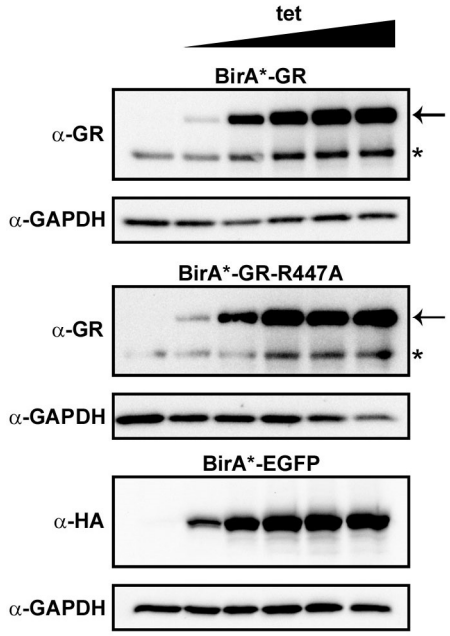
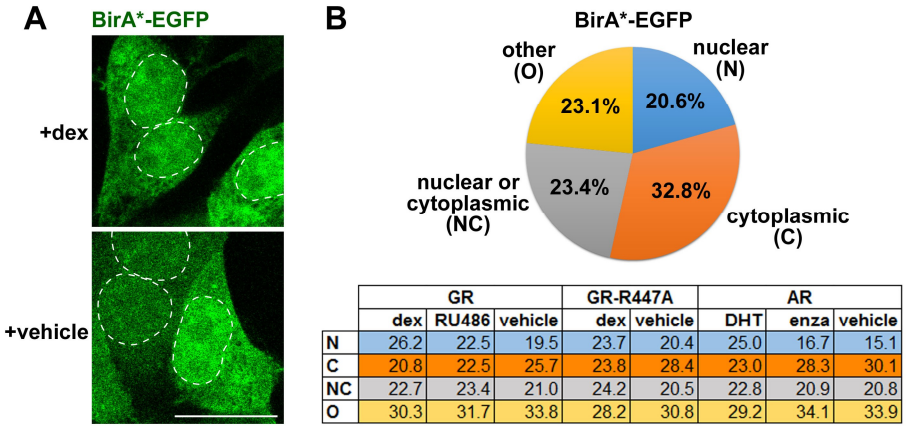
Supplementary Figure 1



Supplementary Figure 1: Characterization of the tetracycline-inducible cell lines expressing BirA* -fused GR, GR-R447A and EGFP. Flp-In 293 T-Rex cells were analyzed 24 h after induction with increasing tetracycline (tet) concentrations (0, 0.01, 0.03, 0.06, 0.1 and 1 µg/ml). Tet concentration 0.03 µg/ml was used for the MS samples. BirA*-GR and BirA*-GR-R447A were detected with anti-GR and BirA*-EGFP with anti-HA. The anti-GR antibody detects low levels of endogenous GR (depicted by asterisks) in addition to the BirA*-fused GR (depicted by arrows).

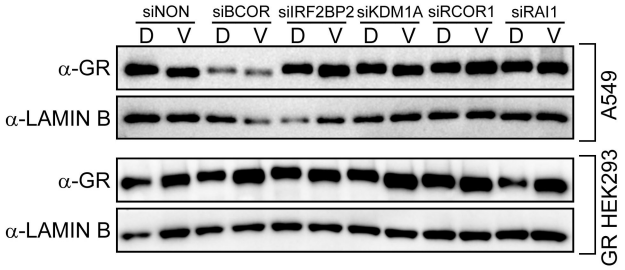
Supplementary Figure 2



	GR			GR-R447A		AR		
	dex	RU486	vehicle	dex	vehicle	DHT	enza	vehicle
N	26.2	22.5	19.5	23.7	20.4	25.0	16.7	15.1
C	20.8	22.5	25.7	23.8	28.4	23.0	28.3	30.1
NC	22.7	23.4	21.0	24.2	20.5	22.8	20.9	20.8
O	30.3	31.7	33.8	28.2	30.8	29.2	34.1	33.9

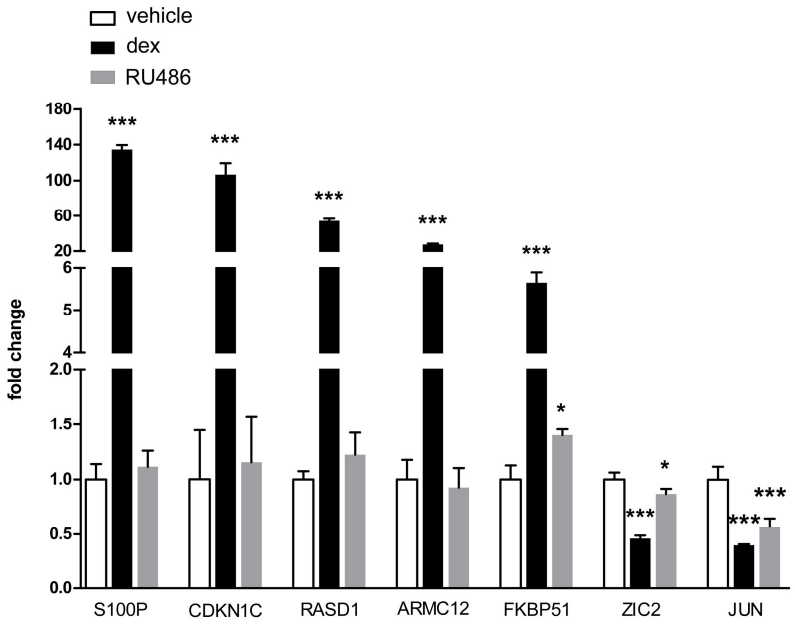
Supplementary Figure 2: Subcellular localization of BirA*-EGFP. (A) Confocal fluorescence microscopy images of BirA*-EGFP-expressing cells treated with 0.03 µg/ml tetracycline and 100 nM dex or vehicle. Dashed lines indicate positions of cell nuclei as inferred from nuclear lamin B staining. Scale bar: 20 µm. **(B)** Pie chart shows the subcellular location of biotinylated proteins in BirA*-EGFP cells as detected by MS. Identified proteins were assigned to subcellular location groups as specified by the Uniprot Subcellular location [CC] attribute, and the spectral count sum for each group was counted. Percentages in the chart depict the spectral count ratio of each group from the total sum. Table shows the same values in percentages for other BirA* samples. Subcellular location of each identified protein is listed in Supplementary Table S2.

Supplementary Figure 3



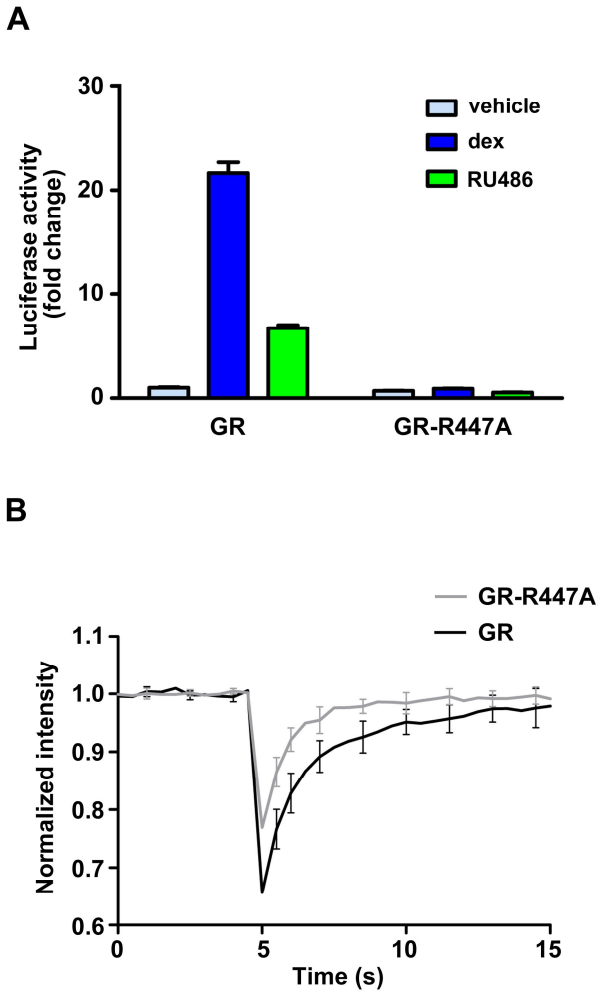
Supplementary Figure 3: GR protein levels after silencing of selected GR interactors. Immunoblots from A549 and GR-expressing HEK293 cells after siRNA silencing of BCOR, IRF2BP2, KDM1A, RCOR1 or RAI1. Cells were transfected with specific siRNAs for 72 h and exposed for 6 h to 100 nM dexamethasone (D) or vehicle (V) before harvesting.

Supplementary Figure 4



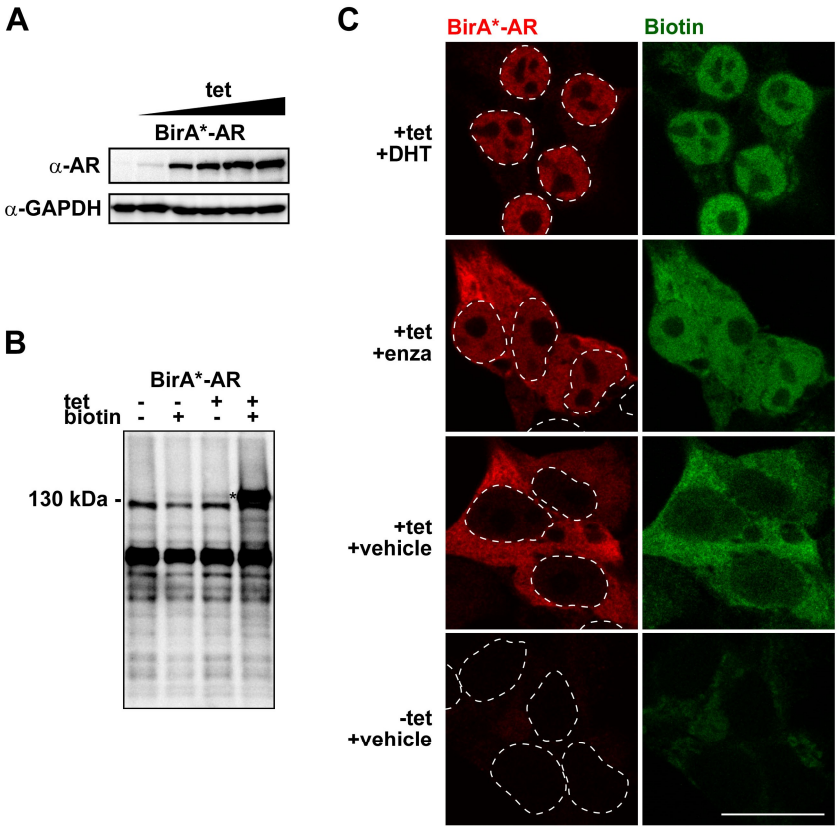
Supplementary Figure 4: Effect of RU486 on GR target gene expression. GR-expressing HEK293 cells were exposed to 100 nM dex, 1 µM RU486 or vehicle for 6 h and mRNA levels were measured with RT-qPCR. Columns represent the mean ± SD of four biological replicates. Values are fold changes compared to vehicle treatment for each gene. Significances are for the difference between vehicle treatment and the corresponding treatment. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ANOVA and Bonferroni.

Supplementary Figure 5



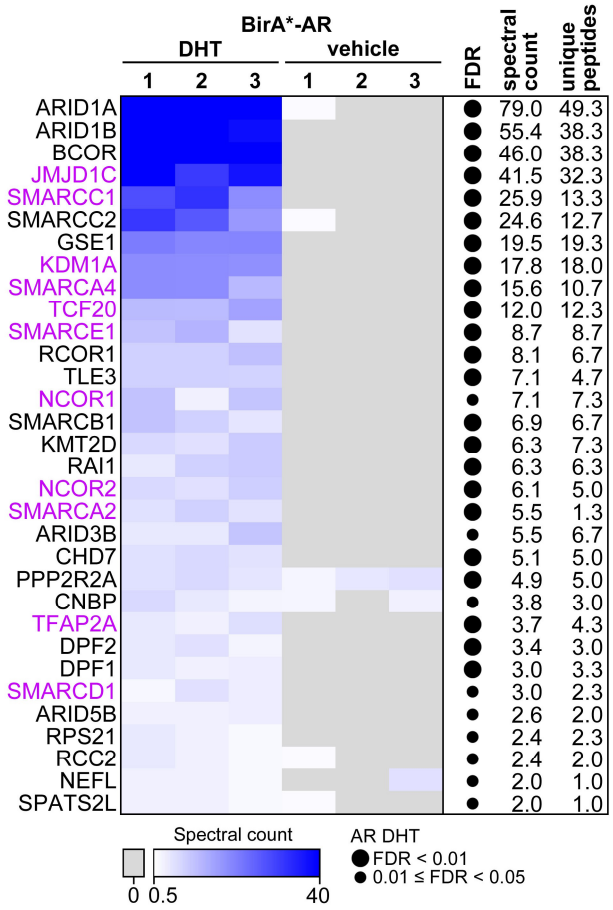
Supplementary Figure 5: Characterization of the GR DNA-binding mutant R447A. (A) Transcriptional activity of GR-R447A was compared to that of GR by luciferase reporter gene assays in the presence of 100 nM dex, 1 µM RU486 or vehicle. COS-1 cells were cotransfected with a minimal pGRE4-tk-luc reporter together with expression vectors encoding GR and GR-R447A. The columns represent mean ± SD of three biological replicates normalized to transfection efficiency using β-galactosidase activity. The activity of GR in vehicle treatment has been set to 1. **(B)** Fluorescence recovery after photobleaching was used to measure the nuclear mobility of GR and GR-R447A. Flp-In T-Rex 293 cells expressing EGFP-GR or EGFP-GR-R447A were exposed to 100 nM dex and a 1-µm wide rectangular area spanning the nucleus was bleached and used to monitor recovery dynamics. Curves represent the mean and error bars ± SD of at least 5 cells.

Supplementary Figure 6



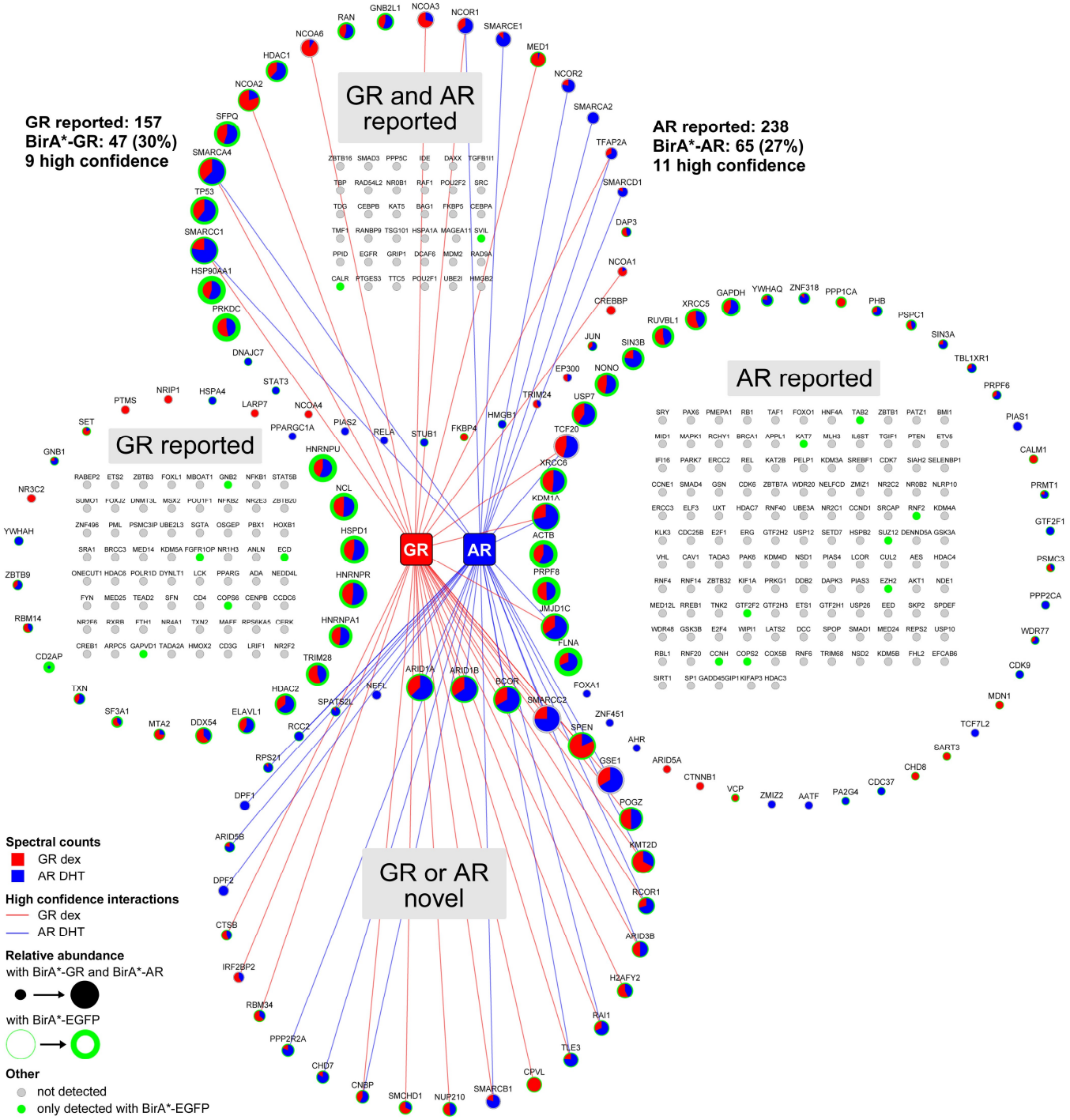
Supplementary Figure 6: Characterization of the BirA*-AR -expressing cell line. (A) Tetracycline-induction of the cell line expressing BirA*-fused AR. Flp-In 293 T-Rex cells were analyzed 24 h after induction with increasing tetracycline (tet) concentrations (0, 0.01, 0.03, 0.06, 0.1 and 1 µg/ml). Tet concentration 0.03 µg/ml was used for the MS samples. **(B)** Immunoblots from cells with (+) or without (-) excess biotin (50 µM) and tet (0.03 µg/ml), detected by streptavidin-HRP. Fusion protein position is depicted by an asterisk. **(C)** Confocal fluorescence microscopy images of BirA*-AR-expressing cells treated with 100 nM DHT, 10 µM enzalutamide (enza) or vehicle (ethanol) in the presence of 50 µM biotin and with or without 0.03 µg/ml tet. BirA*-AR was detected with anti-HA (red) and biotinylated proteins with fluorescently labeled streptavidin (green). Dashed lines indicate positions of cell nuclei as inferred from DAPI DNA staining. Scale bar: 20 µm.

Supplementary Figure 7



Supplementary Figure 7: High confidence interactions identified with BirA*-AR. Heatmap showing the spectral counts of high confidence interactors identified with BirA*-AR. Values for three biological replicates from DHT and vehicle treatment are shown. No high confidence interactions were detected in vehicle treatment. Previously reported AR interactors in the BioGRID (Stark *et al.* 2006, Nucleic Acids Res.; Chatr-Aryamontri *et al.* 2017, Nucleic Acids Res.) database and from individual publications (Metzger *et al.* 2005, Nature.; Mukhopadhyay *et al.* 2006, Exp Cell Res.) are shown in purple. On the right, FDRs, spectral count averages and unique peptide averages are shown for the DHT-treated samples. Spectral counts have been normalized to that of AR in each sample.

Supplementary Figure 8



Supplementary Figure 8: Comparison of the BioID-derived interactomes of the GR and the AR to previously reported interactions. High confidence interactors of the GR with dex and the AR with DHT were compared to the interactions in the BioGRID database (Stark *et al.* 2006, Nucleic Acids Res.; Chatr-Aryamontri *et al.* 2017, Nucleic Acids Res.) and in individual publications (Metzger *et al.* 2005, Nature.; Ebert *et al.* 1998, J. Neurochem.; Mukhopadhyay *et al.* 2006, Exp Cell Res.). Edges represent high confidence interactions identified in this study for the GR (red edge) and the AR (blue edge). Node size shows the relative abundance of each identified protein (average from the GR and AR samples), with the color of the node showing the distribution of the detected spectral counts between the GR (red) and the AR (blue). Green border of the node shows the relative abundance of the protein in BirA*-EGFP samples.