*Suppl. Data*

# NanoLuc Binary Technology as a methodological approach: An important new tool for studying the localization of androgen receptor and androgen receptor splice variant V7 homo- and heterodimers

**Juan Guzman [1,2], Katrin Weigelt [1,2], Angela Neumann [1,2], Philipp Tripal [3], Benjamin Schmid [3], Zoltán Winter [3], Ralph Palmisano [3], Zoran Culig [4], Marcus V. Cronauer [5], Paul Muschler [6], Bernd Wullich [1,2], Helge Taubert [1,2,*,†] and Sven Wach [1,2,†]**

**Suppl. Table S1. List of plasmids used and cloned in this study**

| Name | Gene | Resistance |
|---|---|---|
| pEGFP-AR-WT | Androgen receptor | Kanamycin |
| pEGFP-AR-V7 | Androgen receptor splice variant-7 | Kanamycin |
| pBiT1.1-N[TK/LgBiT] | Cloning NanoBiT Expression Vector | Ampicillin |
| pBiT1.1-C[TK/LgBiT] | Cloning NanoBiT Expression Vector | Ampicillin |
| pBiT2.1-N[TK/SmBiT] | Cloning NanoBiT Expression Vector | Ampicillin |
| pBiT2.1-C[TK/SmBiT] | Cloning NanoBiT Expression Vector | Ampicillin |
| NanoBiT Negative Control | Negative control for Nano-Glo Live Cell Assay System | Kanamycin |
| LgBiT-PRKAR2A Control | Positive control for Nano-Glo Live Cell Assay System | Kanamycin |
| SmBiT-PRKACA Control | Positive control for Nano-Glo Live Cell Assay System | Kanamycin |
| pBiT1.1-N[TK/LgBiT] + AR | NanoBiT construct with the OPF of the Androgen receptor | Ampicillin |
| pBiT1.1-C[TK/LgBiT] +AR-V7 | NanoBiT construct with the OPF of the Androgen receptor splice variant-7 | Ampicillin |
| pBiT2.1-N[TK/SmBiT] +AR | NanoBiT construct with the OPF of the Androgen receptor | Ampicillin |
| pBiT2.1-C[TK/SmBiT] + AR-V7 | NanoBiT® construct with the OPF of the Androgen receptor splice variant-7 | Ampicillin |

**Suppl. Table S2. Workflow of immunofluorescence detection**

| **Timeline** | **HEK-293** |
|---|---|
| Day 0 Coating with Poly-L-Lysine | 500 µL |
| Day 0 Seeding | 200,000 cells |
| Day 1 Transfection | 2 µg DNA |
| Day 2 Stimulation with 1 nM DHT | 2.2 µL |
| Day 2 Fixation with 4 % PFA | 500 µL |
| Day 2 Permeabilization with 0.2% Triton X-100 | 500 µL |
| Day 2 Blocking with 1% BSA + 0.1% Triton X-100 | 500 µL |
| Day 2 Incubation with NanoLuc Luciferase Antibody (1:500) | 1000 µL |
| Day 3 Incubation with the Alexa Fluor 488 antibody (1:1000) | 1000 µL |
| Day 3 Preservation with Mounting Medium containing DAPI | 100 µL |