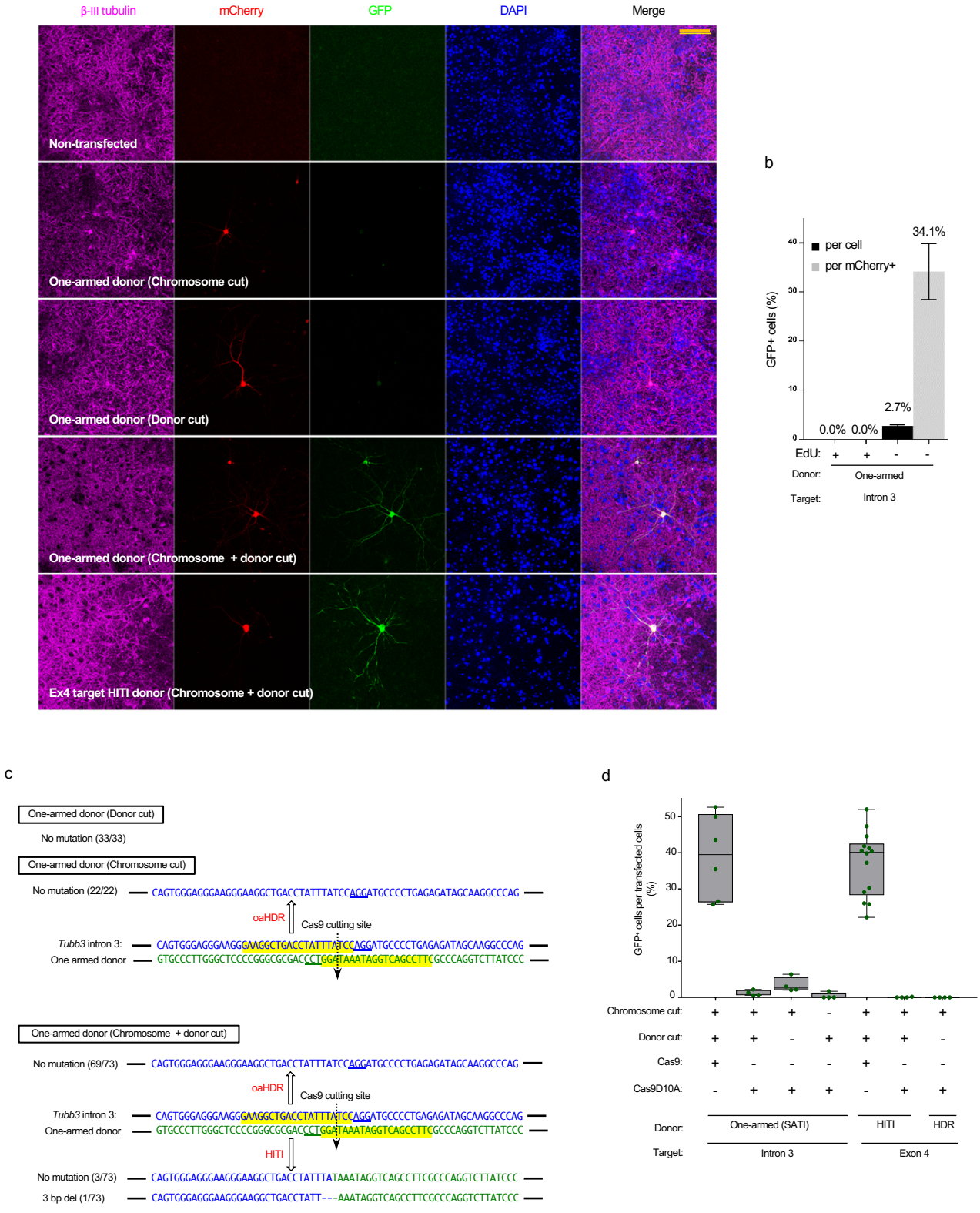
a
β-III tubulin
mCherry
GFP
DAPI
Merge
Non-transfected
One-armed donor (Chromosome cut)
One-armed donor (Donor cut)
One-armed donor (Chromosome + donor cut)
Ex4 target HITI donor (Chromosome + donor cut)
b
per cell
per mCherry+
GFP+ cells (%)
34.1%
2.7%
0.0%
0.0%
EdU:
Donor:
One-armed
Target:
Intron 3
c
One-armed donor (Donor cut)
No mutation (33/33)
One-armed donor (Chromosome cut)
No mutation (22/22)
CAGTGGGAGGGAAGGGAAGGCTGACCTATTTATCCAGGATGCCCCTGAGAGATAGCAAGGCCCAG
oaHDR
Cas9 cutting site
Tubb3 intron 3:
One armed donor
GTGCCCTTGGGCTCCCCGGGCGCGACCCTGGATAAATAGGTCAGCCTTCGCCCAGGTCTTATCCC
One-armed donor (Chromosome + donor cut)
No mutation (69/73)
HITI
No mutation (3/73)
3 bp del (1/73)
CAGTGGGAGGGAAGGGAAGGCTGACCTATTTATAAATAGGTCAGCCTTCGCCCAGGTCTTATCCC
CAGTGGGAGGGAAGGGAAGGCTGACCTATTT---AAATAGGTCAGCCTTCGCCCAGGTCTTATCCC
d
GFP+ cells per transfected cells (%)
Chromosome cut:
Donor cut:
Cas9:
Cas9D10A:
Donor:
One-armed (SATI)
HITI
HDR
Target:
Intron 3
Exon 4

Supplementary Figure S4 | Development of novel targeted gene knock-in method in primary neurons. a Representative pictures of non-transfected and transfected neuronal cultures with the different donors and gRNAs for recognizing the cutting patterns induced by one arm homology and HITI donors. Images were acquired with confocal microscopy using 20x objective, scale bar: 100 μm. **b** Absolute and relative knock-in efficiency indicated by the percentage of GFP+ cells among total cells (DAPI+) or transfected cells (mCherry+) in EdU+ or EdU- neurons. $n = 7$. Each value indicates percentage of GFP positive cells among total cells (black) or transfected cells (light gray). Data are represented as mean ± s.e.m. **c** An example of actual sequence after GFP knock-in at the 3' end of the *Tubb3* coding region via one homology arm donor (MC-Tubb3int3-SATI). Broken arrow, Cas9 cutting site. Underlined sequence corresponds with PAM sequence. Yellow highlight is indicated gRNA sequence. Sequence indicated as green is inserted sequence derived from donor vector. Sequence indicated as blue is targeted genomic sequence. **d** Effect on the efficiency of GFP knock-in in neurons by comparison of wild-type Cas9 (Cas9) and Cas9 nickase (Cas9D10A, introducing a single-strand break) in SATI donors (MC-Tubb3int3-SATI, MC-Tubb3int3-scramble), HITI donor (Tubb3ex4-HITI) and HDR donor (Tubb3ex4-HDR). Data are represented as box with whiskers including all input data points as green dots, average in the middle of the box.