Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/7/eaaw1888/DC1

# Supplementary Materials for

## Exercise training improves motor skill learning via selective activation of mTOR

Kai Chen, Yuhan Zheng, Ji-an Wei, Huan Ouyang, Xiaodan Huang, Feilong Zhang, Cora Sau Wan Lai, Chaoran Ren, Kwok-Fai So*, Li Zhang*

*Corresponding author. Email: hrmaskf@hku.hk (K.-F.S.); zhangli@jnu.edu.cn (L.Z.)

Published 3 July 2019, *Sci. Adv.* **5**, eaaw1888 (2019)
DOI: 10.1126/sciadv.aaw1888

**This PDF file includes:**

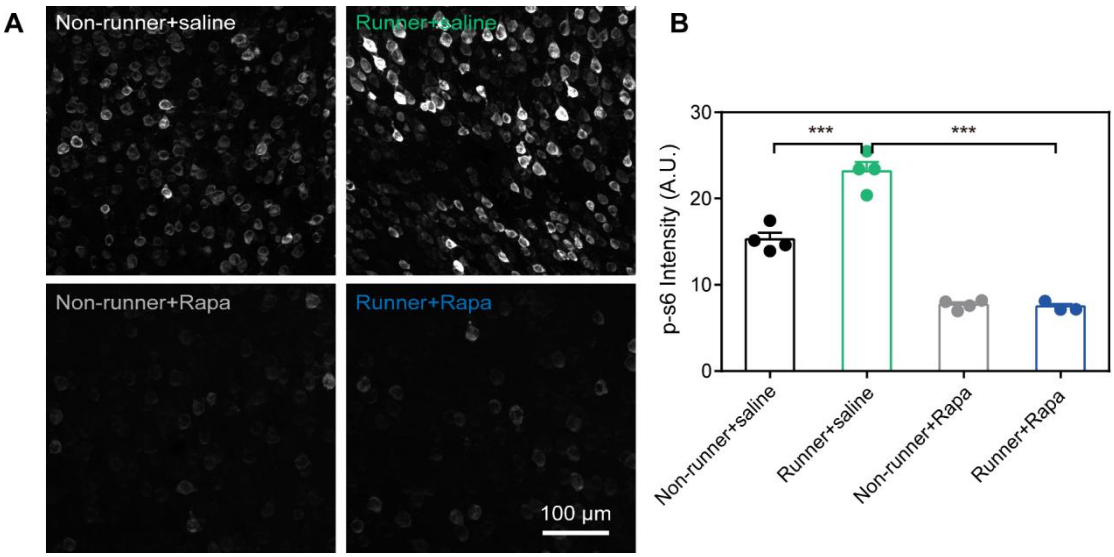


**Fig. S1. Exercise-activated ribosomal protein S6 in motor cortex.** (A) Representative images of immunofluorescent staining showing prominently higher levels of phosphorylated S6 (p-S6) in motor cortex. (B) Quantification of p-S6 fluorescent intensity among four groups (Non-runner: $n$=4; Runner: $n$=4; Non-runner + Rapa: $n$=4; Runner + Rapa: $n$=3; One-way ANOVA, $F_{3,11}$=106.1, $P$<0.0001). Runner mice showed significantly higher p-S6 levels than non-runner controls (Tukey's comparison, $q_{11}$=11.24, $P$<0.0001), and rapamycin injection suppressed p-S6 level ($q_{11}$=20.67, $P$<0.0001). ***, $P$<0.001. Error bar, s.e.m.

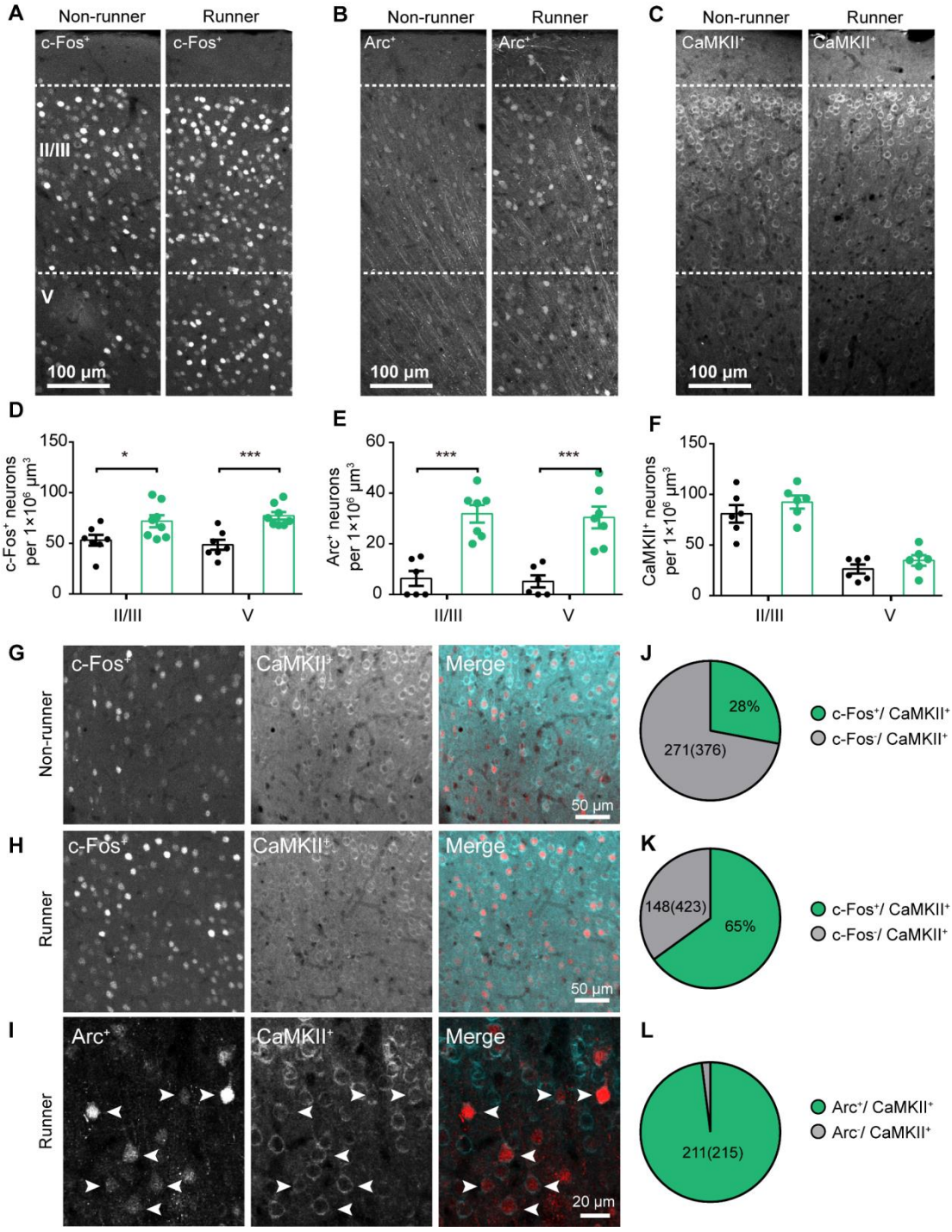


**Fig. S2. Exercise-induced neuron activity of cortical pyramidal neurons.** (A to C) Representative immunofluorescent staining images showing the expression of c-Fos (A), Arc (B) and CaMKII (C) in coronal sections of motor cortex between non-runner ($n$=7) and runner mice ($n$=8). (D to F) Quantification of positive stained cells in layer II/III and layer V. Runner mice had significantly more c-Fos$^+$ neurons comparing to non-runner controls (Unpaired t-test, Layer II/III: $t_{13}$=2.317, $P$=0.0374; Layer V: $t_{13}$=4.669, $P$=0.0004; D), and higher levels of Arc expression

(Unpaired t-test, Layer II/III: $t_{11}$=5.483, $P$=0.0002; Layer V: $t_{11}$=4.921, $P$=0.0005; E), whilst no change of CaMKII$^+$ cell number existed (Unpaired t-test, Layer II/III: $t_{10}$=1.059, $P$=0.3145; Layer V: $t_{10}$=1.251, $P$=0.2393; F). (G to I) Representative imaging planes for cFos and CaMKII co-labelling in Layer V of motor cortex in non-runner (G) and runner mice (H), and for Arc and CaMKII co-labelling (I). (J to L) Cell counts showed more cFos$^+$ cells in CaMKII$^+$ neurons after exercise training, and almost all Arc$^+$ cells are CaMKII$^+$ neurons. *, $P$<0.05; ***, $P$<0.001. Error bar, s.e.m.

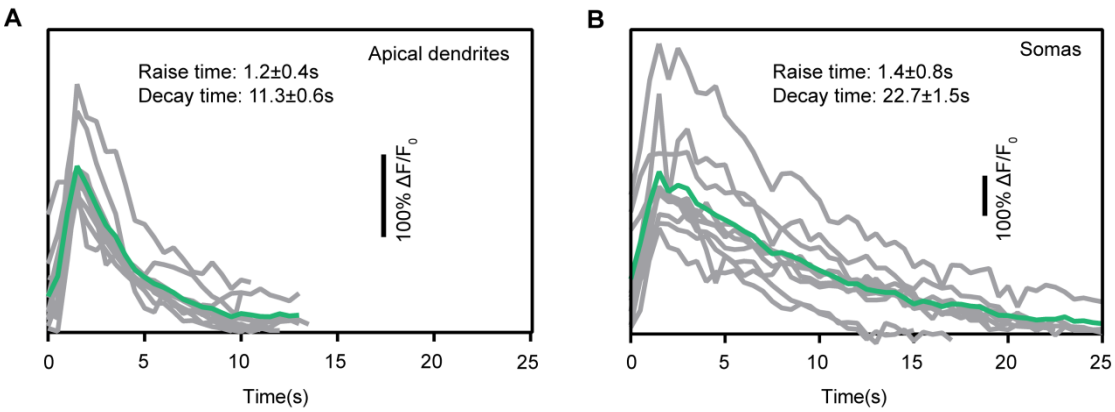


**Fig. S3. Calcium spike dynamics of apical tuft and soma of L5PRN.** Representative recording traces (gray) from controlled mice were overlaid with averaged trace (green). In general, calcium spike in soma showed similar rise time but longer decay time comparing to those in apical tuft.

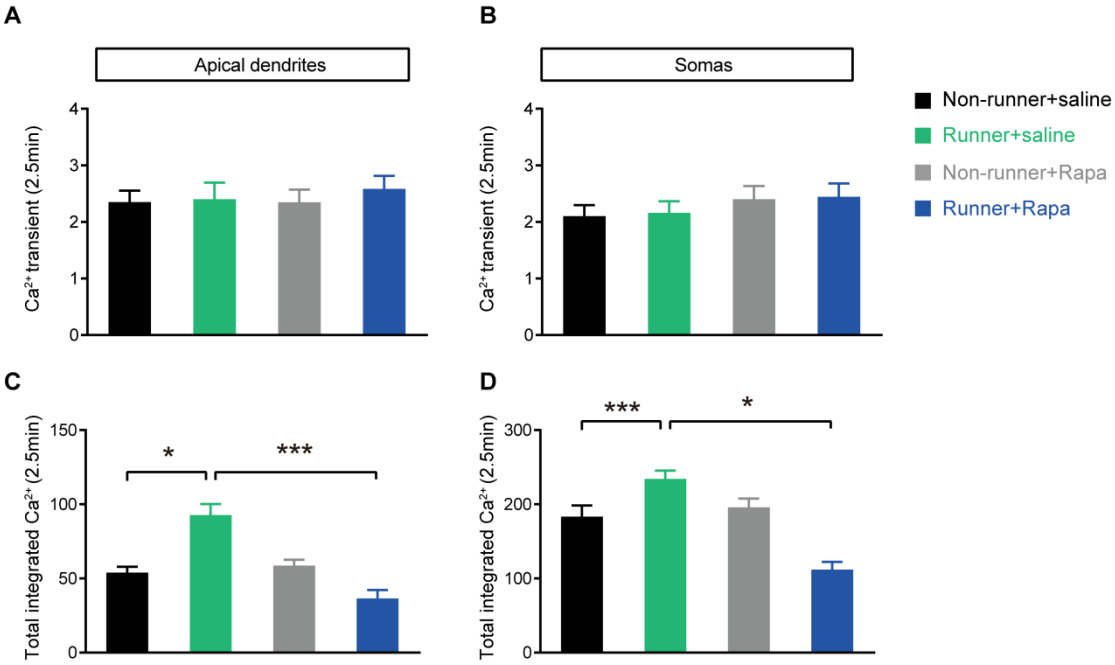


**Fig. S4. Calcium transients of L5PRN.** (A) Quantification of $Ca^{2+}$ transients within a 2.5-min recording trace in apical dendrites. (B) Quantification of $Ca^{2+}$ transients in somas. No significant change was found in the frequency of calcium transient (Apical dendrite: Kruskal-Wallis test, $P$=0.5523; Soma: $P$=0.6453). (C) Total integrated $Ca^{2+}$ levels within a 2.5-min recording trace in apical dendrites. (D) Total integrated $Ca^{2+}$ levels in apical somas. Runner mice ($n$=6) showed significantly higher total integrated $Ca^{2+}$ (Apical dendrite: $F_{3,119}$=15.72, $P$<0.0001; $q_{119}$=7.796, $P$<0.0001; Soma: $F_{3,335}$=12.69, $P$<0.0001; $q_{335}$=4.195, $P$=0.0169) than non-runner mice ($n$=4). Such enhancements were further abolished by rapamycin injection. *, $P$<0.05; ***, $P$<0.001. Error bars, s.e.m.

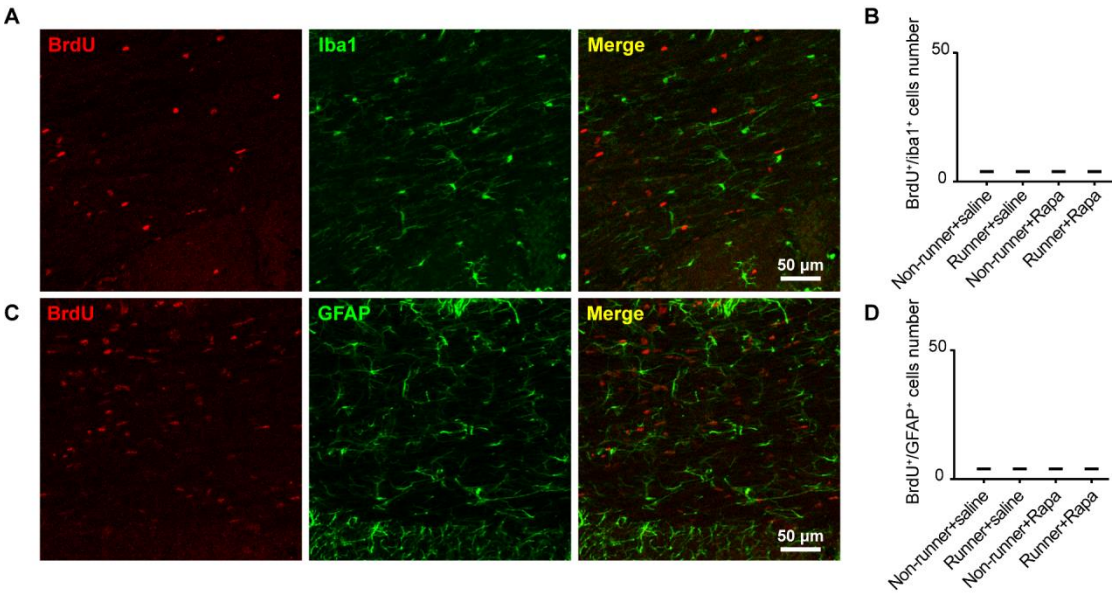


**Fig. S5. Subtyping of BrdU$^+$ cells in the CC region.** Double fluorescent labelling showed barely any microglial cells (A and B) or astrocytes (C and D) that were BrdU$^+$ cells.

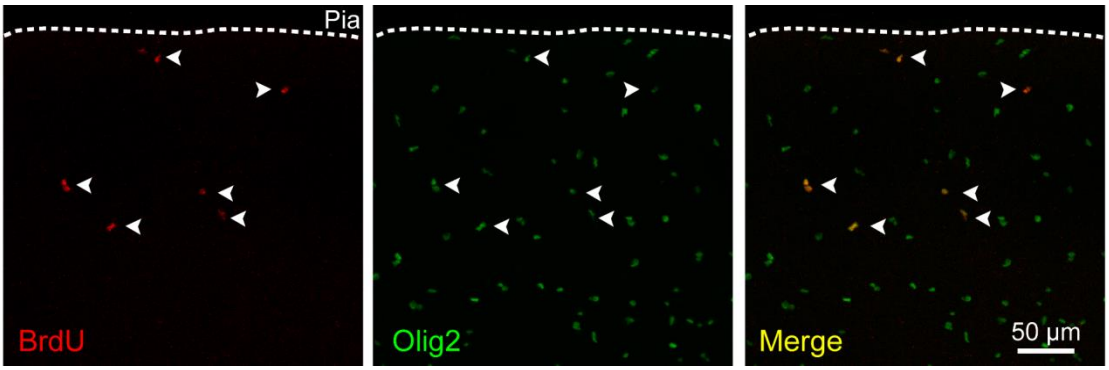


**Fig. S6. Immunofluorescence labeling of BrdU and Olig2 in mouse motor cortex after exercise.** Most of BrdU$^+$ cells were OLs.

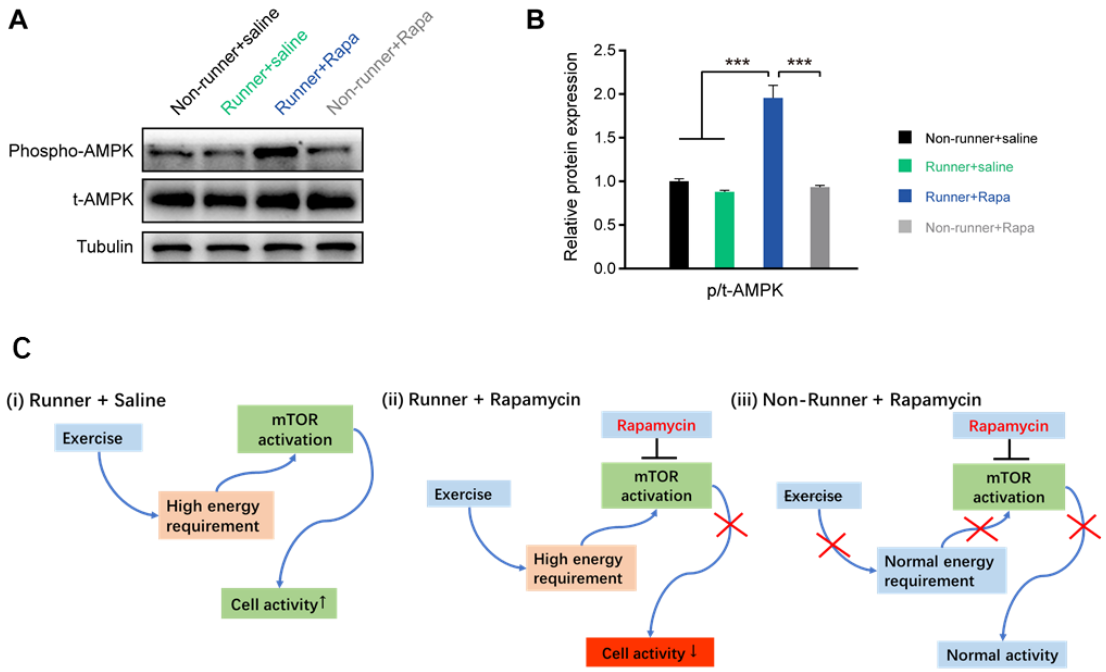


**Fig. S7. Energy requirement of cells under exercise.** (A) Representative Western blotting bands and (B) Quantitative analysis of p-AMPK in mouse motor cortex. Exercise + rapamycin mice presented significantly higher p-AMPK level than other groups ($F_{3,12}$=184.8, $P$<0.0001). $n$=4 per group. (C) A proposed working model for exercise, mTOR and cell activity. Rapamycin can suppress the exercise-activated mTOR pathway, making it unable to satisfy energy requirements of cells under exercise paradigms. In non-runner mice, however, mTOR suppression may not affect basal levels of cell activity due to the normal energy requirement, which does not require mTOR activation.

**Table S1. Primary and secondary antibody used in Western blotting and immunofluorescence staining.**

| | Primary antibody | Host | Company | Dilution | Catalog # |
|---|---|---|---|---|---|
| Western blotting (WB) | BDNF | Mouse | Millipore | 1:1000 | MABN114 |
| | Phospho-TrkB (Tyr816) | Rabbit | CST | 1:1000 | 4168 |
| | Trkb | Rabbit | CST | 1:1000 | 4603 |
| | Phospho-AKT (Ser473) | Rabbit | CST | 1:1000 | 4060 |
| | AKT | Rabbit | CST | 1:1000 | 9272 |
| | Phospho-mTOR (Ser2448) | Rabbit | CST | 1:1000 | 2971 |
| | mTOR | Rabbit | CST | 1:1000 | 2972 |
| | Phospho-s6 (Ser240/244) | Rabbit | CST | 1:1000 | 2215 |
| | s6 | Rabbit | CST | 1:1000 | 2217 |
| | 4E-BP2 | Rabbit | CST | 1:1000 | 2845 |
| | PSD95 | Rabbit | CST | 1:1000 | 3450 |
| | Synaptophysin | Rabbit | CST | 1:1000 | 5461 |
| | SNAP25 | Rabbit | CST | 1:1000 | 5308 |
| | Actin | Rabbit | CST | 1:1000 | 8457 |
| | β-Tubulin | Rabbit | CST | 1:1000 | 2146 |
| | Phospho- AMPK (Ser485) | Rabbit | CST | 1:1000 | 4184 |
| | AMPK | Rabbit | CST | 1:1000 | 2532 |
| Immuno-fluorescence (IF) | Phospho-s6 (Ser220/244) | Rabbit | CST | 1:500 | 2215 |
| | c-Fos | Guinea pig | SY SY | 1:500 | 226004 |
| | Arc | Rabbit | SY SY | 1:1000 | 156003 |
| | CaMKII | Rabbit | Abcam | 1:250 | ab5683 |
| | Myelin Basic Protein | Rabbit | Abcam | 1:500 | ab40390 |
| | BrdU | Rat | Abcam | 1:500 | ab6326 |
| | Olig2 | Rabbit | Abcam | 1:500 | ab109186 |
| | CC1 | Mouse | Calbiochem | 1:20 | OP80 |
| | PDGFRalpha | Goat | R&D systems | 1:500 | AF1062 |
| | Ki67 | Rabbit | Abcam | 1:500 | ab15580 |
| | Iba1 | Rabbit | Wako | 1:1000 | 019-19741 |
| | GFAP | Mouse | Millipore | 1:500 | MAB360 |
| Secondary antibody (WB) | Goat Anti-Rabbit IgG H&L (HRP) | - | Abcam | 1:5000 | ab6721 |
| | Rabbit Anti-Mouse IgG H&L (HRP) | - | Abcam | 1:5000 | ab6728 |
| Secondary antibody (IF) | Goat Anti-Rabbit (Alexa Fluor® 488) | - | Abcam | 1:500 | ab150077 |
| | Goat Anti-Rabbit (Alexa Fluor® 594) | | Abcam | 1:500 | ab150080 |
| | Goat Anti-Mouse (Alexa Fluor® 488) | - | Abcam | 1:500 | ab150113 |
| | Rabbit Anti-rat (Alexa Fluor® 555) | - | CST | 1:500 | 4417 |
| | Goat anti-Guinea Pig (Alexa Fluor® 488) | - | Invitrogen | 1:500 | A-11073 |
| | Donkey anti-Goat (Alexa Fluor® 488) | - | Invitrogen | 1:500 | A32814 |