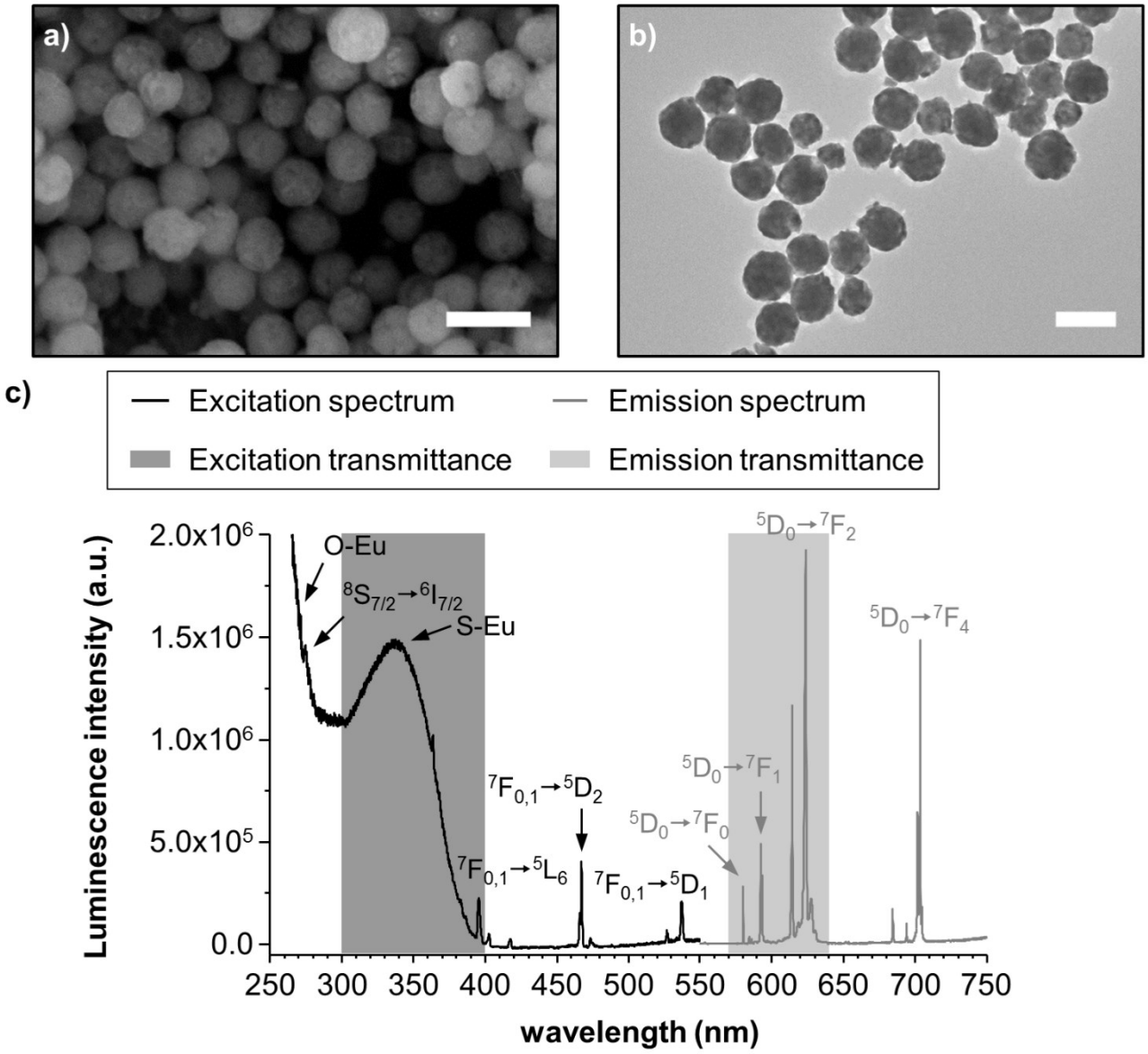

Fig. S1 Characteristics of $Gd_2O_2S:Eu^{3+}$ NPs. Representative a) Scanning and b) Transmission electron micrographs of the NPs after synthesis (scale bar 200 nm). c) Luminescence spectra. Excitation spectrum monitored at 624 nm and emission spectrum under a 365 nm excitation source. Transmittance windows used for fluorescence microscopy are shown in transparence.

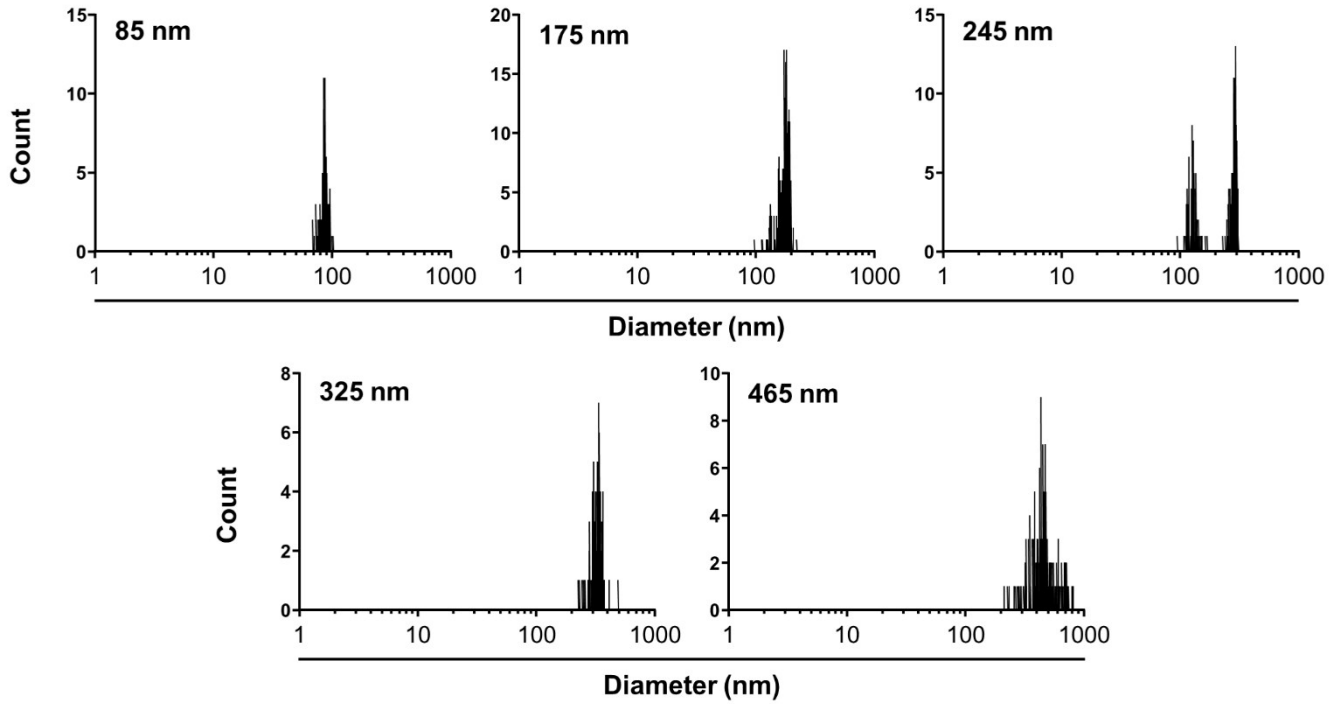

Fig. S2 Nanoparticle diameters of different batches measured with TEM (mean measured over at least 100 different NPs)

Fig. S3 Videomicroscopy acquisition of $Gd_2O_2S:Eu^{3+}$ labeling of MSCs

S4: Photophysical considerations about $Gd_2O_2S:Eu^{3+}$ NPs

Excitation properties

The excitation spectrum of $Gd_2O_2S:Eu^{3+}$ NPs is presented in Figure S1. Several excitation bands can be observed:

The first one, located in the deep UV range (below 250 nm), is very efficient and common to many europium-based materials such as oxides or carbonates (much more studied for decades) and corresponds to the $O^{2-} \rightarrow Eu^{3+}$ ions charge transfer. However, this wavelength range is known to be very deleterious for living cells. To overcome this problem europium doped gadolinium oxysulfide NPs have been developed, which allow a red shifted excitation wavelength (shift towards the end of the visible spectrum). Indeed, on the excitation spectrum, an intense excitation band centered at 340 nm can be observed, corresponding to the $S^{2-} \rightarrow Eu^{3+}$ ions charge transfer. The presence of 360 nm excitation filters on fluorescence microscopes and flow cytometers is rather common; therefore, this $S^{2-} \rightarrow Eu^{3+}$ charge transfer can be easily used to efficiently visualize $Gd_2O_2S:Eu^{3+}$ NPs.

It is also possible to directly use Eu^{3+} ions for excitation of $Gd_2O_2S:Eu^{3+}$ NPs instead of charge transfer bands although all the corresponding peaks are much less intense. Several absorption peaks can be observed in the visible region, corresponding to $^7F_{0,1} \rightarrow ^5L_6$ and $^7F_{0,1} \rightarrow ^5D_{J(J=1-3)}$ transitions of Eu^{3+}. Consequently, 395, 465 or 537 nm excitation wavelengths can be used. In addition, 405 and 488/514 nm excitation wavelengths can also be used; however, absorption peaks are even less efficient. The NPs brightness obtained using those peaks will be 10 or 100 times lower than exciting in the near UV range. Therefore, obtaining equivalent intensities would require a powerful excitation source (laser or lamp). Nevertheless, using a confocal microscope (Leica Model SP5) equipped with argon laser and a femtosecond-pulsed Ti: sapphire laser (Libra II, Coherent), it has been demonstrated to work properly.[40]

Finally, $Gd_2O_2S:Eu^{3+}$ NPs also exhibit two-photon excitation properties with an excitation wavelength centered at 720 nm that allows the visualization of the 625 nm peaks ($^5D_0 \rightarrow ^7F_2$ transition). However, here again, the emission intensity is low and obtaining equivalent intensities requires very powerful laser excitation. Moreover, if two photon excitations is preferred, it is much more efficient to use Gd_2O_2S doped with the couple Yb8%/Er1% as already published by us.[11]

Quantum efficiency and Brightness

Brightness can be defined as the parameter characterizing fluorescence intensity. Quantum efficiency and brightness are linked by the following relation:

$$B = \alpha.\varepsilon.\Phi$$

Where B is the brightness, Φ the quantum yield, ε the molar extinction coefficient and α a coefficient linked to the chromophore number (amount of chromophore units: dyes or luminescent centers).

For molecular dyes, ε and Φ remain constant over a wide range of dye concentration, and thus collected intensity is directly proportional to Φ and concentration. As a consequence, the higher the Φ is, the better the dye.

For nano-objects containing luminescent centers, which are strongly confined environments, quantum yield rapidly decreases when luminescent centers concentration increases (self-quenching effect). The opposite is true for absorption, which usually increases with the concentration. It is thus necessary to find the right compromise and the key parameters to maximize the brightness B, which is not obtained at quantum yield maximum. Quantum yield is moreover very difficult to determine precisely when working with nanoparticles because of the NPs diffusion which strongly disturbs absorption measurements. This explains why the quantum yield of this type of mineral nano-phosphors is rarely reported in the literature: it is difficult to measure and depends on many parameters like the concentration or the surface hydration state. Gd_2O_2S: Eu^{3+} (5%). NPs presented in this paper have consequently been optimized to maximize the brightness.

Quantum yield has been evaluated by us to be around 50% for $Gd_2O_2S:Eu^{3+}$ (5%) NPs. This value can appear to be low compared to that of molecular dyes (Φ_{FITC}=90%), but thanks to the extreme confinement of Eu^{3+} luminescent centers inside the NPs (several billions in one particle of 150 nm), the brightness becomes several orders of magnitude higher than any molecule.
It is also important to keep in mind that this sort of mineral nanophosphors have three other advantages compare to molecular dye:

1- They are not subject to photo-bleaching; allowing very powerful excitation over a very long period of time.
2- The Stokes shift (gap between excitation and emission wavelength) is huge; allowing easy excitation beam separation.
3- The fluorescence life time is (very) long; allowing time gated detection (eliminating all biological media auto-fluorescence). Readers interested in this aspect of Time Gated Luminescent Microscopy can advantageously consult the paper of *Zhang et al.* [41]

360 nm UV excitation and photo-toxicity

As reported by *Ge et al.*[42], 360 nm UV excitation is genotoxic for living cell while excitation in the visible area beyond the blue (> 460nm) is not. The same authors report that a 5 minutes excitation under a microscope at 360nm can induce detectable DNA damages which reach 20% after 15 minutes. However, the observation of $Gd_2O_2S:Eu^{3+}$ NPs presented in this paper was conducted with a classical epifluorescence microscope equipped with an HXP lamp and an exposure time of 1.5 seconds was amply sufficient to detect strong fluorescence.
In addition, if visible radiations are intrinsically not toxic to living cells, many molecular dyes become photo-toxic under visible excitation which also limit much their use on living cells.
As a result, one can prefer to use a powerful excitation source at 488/514 nm instead of 365 nm (see subsection above) to avoid any damage to the living cells. However, the toxicity of the excitatory radiation is truly inconvenient only if it is desired to re-use the labeled cells that are observed by microscopy or flow cytometry. This is not usually the case. Indeed, microscopic observation or flow cytometry analysis has two main interests in our sense:

- 1: Evaluate the amount of particles internalized inside the cells to ensure their correct labeling. This evaluation is made on very small sample quantities compared to that of labeled cells which will then be injected for them *in vivo* tracking.
- 2: Locate these labeled cells on *post-mortem* histological sections. In this case, the sample is fixed and therefore the cells are already dead.

To summarize, 365 nm excitation of $Gd_2O_2S: Eu^{3+}$ NPs is to be used for flow cytometry or classical fluorescence microscopy. 488/514 or even 720 nm excitation band can be used using a confocal or multiphoton microscope equipped with powerful lasers for *intravital* microscopy for instance. Animal whole body photo-imaging cannot be performed using $Gd_2O_2S:Eu^{3+}$ NPs but is possible using $Gd_2O_2S:$ Yb8%, Er1% (or Tm) (under development).